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PRIMARY SPLENOMEGALY.

WITH A REPORT OF THREE CASES OCCURRING IN ONE FAMILY.[1]

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THE cases which are about to be reported here are, in addition to their extreme rarity, noteworthy because their subjects have been under my observation for over fifteen years. I have refrained from publishing them earlier, since no scientific results could be obtained from a mere description of their clinical histories. I had long considered them unique and knew of no other cases in the literature just like them. They are now recorded so as to supplement the valuable contribution to *splenomegalia primitiva* made by David Bovaird, Jr., in a recent number of THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES (October, 1900), in which he describes two similar cases.

These cases reveal a family form of primary splenomegaly all developed in one generation. On this account the *family history* of these patients is important.

The mother of my patients is in her sixty-second year, in perfect health. The father died last year, under my care, of pneumonia. Of the maternal parents (the grandparents of the patients), the father died at seventy-two years, likewise of pneumonia, under my observation; the mother at thirty-eight years, in childbirth. Of the paternal parents, the father is said to have died of gallstones at seventy-three years; the mother died last year, at ninety-four years, of shock due to a fall down stairs, while under my care. In none of them who were under my observation was there any enlargement of the spleen or liver.

As far as inquiry as to the great-grandparents of our patients goes, an accurate report can be furnished only as to those on the maternal

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[1] Two of the patients were presented at the meeting of the Society of Alumni of Bellevue Hospital, November 7, 1900.

The changes which take place in the muscle-fibres as the result of the invasion of muscle tissue by the tumor are manifold. All the possible degenerative changes which occur in the various lesions of muscle may be present, and they may occur very irregularly in the individual fibres. The commonest form is simple atrophy, but the most interesting are (*a*) the multiplication of the nuclei, (*b*) the irregular or ampullar atrophy, and (*c*) the formation of giant-cells.

(*a*) Ordinarily the muscle nuclei disappear as the muscle-fibre atrophies. In other cases, however, they appear very active, increasing greatly in number (the writer counted 153 in one fibre), chiefly, perhaps exclusively, by direct division. They may form single or double rows, or (*c*) may collect in clumps which on cross section appear like giant cells.

(*b*) Sometimes the tumor cells press against the side of the muscle-fibres causing irregular atrophy which gives an ampullar or beaded appearance to the muscle-fibres.

One point to be insisted on is that all the changes which occur in regenerating muscle, and which are looked upon as regenerative in nature, occur practically in the same form at the periphery of a malignant growth where there can be no question of regeneration. Apparently the muscle-fibre can show at a certain stage of regressive change an appearance similar, or nearly similar, to that seen at the beginning of its development; it is necessary, therefore, to guard against judging from microscopical examination only whether regeneration or degeneration is present.

In many cases there occurs at the edge of the tumor, as the result of reaction on the part of the tissues, a change in the interstitial tissue of the muscle due to round-cell infiltration, growth of the connective tissue, and growth of the intima of the vessels. These all exert an effect on the muscle-fibres. A round-cell infiltration of the perimysium at the edge of the tumor is, as a rule, more frequent and more marked with carcinoma than with sarcoma.

Two conclusions drawn by the writer cannot be accepted without further proof, namely, that cell formations may arise from altered muscle-fibres and take part in the formation of tumor-cells, and that the muscle-fibres may be converted into connective tissue.

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We next discovered that we could crack their edges with the cover-glass. There was no reason, even then, to believe that these might not be unusual forms of fat. We next injected them into the abdominal cavities of animals. Most of the animals developed peritonitis, and large quantities of these bodies could be obtained from the peritoneal fluid. In the last few days we have observed the round form develop under the cover-glass. They can be found in every cancer if properly sought for, and can be injected into animals and be recovered. They also change their form.

"We would not have made these statements were it not for a remarkable experiment at the beginning of this work. Dr. Park had a case of abdominal carcinosis. An exploratory incision was made, and a quantity of the serum from the abdominal cavity was given to me for investigation. It was in this fluid that we first found these bodies, and observed that they did not increase. After the fluid had been kept in the thermostat for three weeks we could still find the same bodies. They were then injected into the jugular vein of a guinea-pig, and three weeks and a half afterward, on killing the animal, we found a primary adenocarcinoma of the lung. This experience, we may say, is unique. The fluid had been carefully sedimented, and the fluid for inoculation taken from the top, so that it was not at all probable that cancer cells were actually introduced into the animal. In the case from which the fluid was taken the growth was a colloid carcinoma, but this was not the form of cancer which developed in the inoculated animal. It is also to be noted that carcinoma in the guinea-pig is extremely rare."

Since the period of this statement our attention has been principally turned to an investigation into the nature of these parasites, in attempts to demonstrate them in the tissue, and in attempts to cultivate them. The following is a detailed report of the experiment:

CASE I.—S. N., a male, aged fifty-one years, a patient of Dr. Park. He was seen at his home, and presented physical signs of a large intraperitoneal tumor. The patient was removed to the Buffalo General Hospital, where an exploratory operation was performed by Dr. Park on August 12, 1898. Preparations had previously been made to collect aseptic fluid in sterile tubes and flasks. Dr. Park opened the distended abdomen with the usual aseptic precautions, and a sterile tube was inserted in the opening and about half a litre of the fluid was permitted to flow into a flask. Then three test tubes in succession were held beneath the running tube, and the remainder of the fluid was drawn off in separate flasks. The fluid which was removed from the peritoneal cavity was slightly blood-stained, but did not coagulate. The three tubes, labelled *a*, *b*, and *c*, were immediately placed in the writer's inside vest-pocket and were carried warm to the laboratory, where they were placed in the thermostat. The flasks were not treated in this manner, but were allowed to fall to the room-temperature and were not incubated. Inoculations upon bouillon, sugar-water, and acid sugar-water, as well as agar and blood-serum, were at once made from all three tubes. A careful microscopical examination was likewise made of the peritoneal fluid, which was found to contain a few blood-corpuscles and some pale spherical bodies, in size varying from 2 to 10 micromillimetres. They were homogeneous, of pale, yellowish-green color, and at first were mistaken for fat droplets, although on closer examination their refractive index was seen to be too low. They were likewise resistant to ether, even when the fluid or tissues containing them were first treated with potassium hydrate, then centrifuged, washed in water, treated with 60, 80, 95 per cent., and absolute alcohol, washed in absolute alcohol and ether, equal parts, and then placed in an ether extractor for four days. When treated with osmic acid they failed to give the black reaction which characterizes fat. Attempts to stain them were partly successful, and it was found with cover-slips fixed by heat that although the bodies were greatly deformed by the process a certain number of them could be stained with carbol-thionin or the usual aniline dyes. Some of these bodies, though not very numerous, contained granular material, which showed marked Brownian movement. The tubes were examined from day to day, and we were able to demonstrate that these spherical bodies gradually increased in size, became more indefinite, and gradually lost their yellowish-green color. As they increased in size they apparently became more fluid and commonly sent out pseudopods and long projections. Fine colorless granules appeared in the protoplasm, and in some a delicate nucleus could be made out. Ultimately the larger forms of the organism became transformed into what appeared to be large sacs containing highly refractive granules and small spherical bodies, not unlike the free spherical bodies above described. The membrane of the sac was demonstrable as a pale colorless structure. The diameter of these sacs was on the average about 20 to 25 micromillimetres, or the size of a good-sized epithelial cell.

On August 19th 3 c.c. of tube *a* were injected into the peritoneal cavity of a female dog. On August 20th a fine surface growth was noted in tubes *a* and *b* of a small organism about the size of a coccus, which presented a peculiar bud-like growth and which resisted all attempts at cultivation. On August 25th 3 c.c. of fluid from tube *c* were injected into the jugular vein of a full-grown guinea-pig (Pig 1). On August 26th 4 c.c. of the same tube were injected into the peritoneal cavity of a second guinea-pig (Pig 2).

On August 29th the patient, who since the operation had not been doing well, died. Unfortunately the autopsy was begun by the attending physician, who opened the abdominal cavity and had already contaminated its contents before the peritoneal fluid was collected.

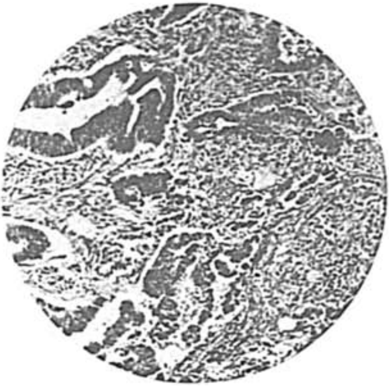
The following is extracted from the autopsy notes:

Autopsy four hours after death. Body of a well-formed, middle-sized man. Muscular structure well developed, skin pale, fat scanty. Peritoneal cavity contains a large amount of serosanguineous fluid. The peritoneum is greatly thickened, contains a large number of translucent vesicles, which are likewise scattered over the entire peritoneal surface, including that of the liver and the under surface of the diaphragm. The pelvic cavity is entirely filled with these gelatinous masses. On removing the enlarged omentum it is found to measure 95 cm. in the long axis. Its greatest thickness is 12 cm. After removing the omental tumor the intestines are apparently free, except a large encapsulated mass of gelatinous material near the ileocæcal junction, apparently involving the lumen of the intestine. Intestines are removed without difficulty, when the tumor in the neighborhood of the cæcum is found to be firmly attached to the peritoneal wall, is apparently of a cystic nature, and contains a mass of gelatinous material. On opening the intestine no connection between the lumen of the cæcum and the tumor mass can be detected. The tumor mass, however, involves the appendix, which is obliterated. The spleen is dark red in color, and weighs four ounces. The right kidney is embedded in a mass of gelatinous material. The capsule of the kidney strips easily, the diameter of the cortex is 5 mm., of grayish-rose color. The papillæ are anæmic. Kidney pelvis unchanged. Left kidney same as right. The gelatinous material in the pelvis fills in the space between the rectum and bladder. The entire under surface of the diaphragm is thickly infiltrated with gelatinous vesicles. The liver is adherent to the diaphragm, and the suspensory and round ligaments are thickly infiltrated with gelatinous material. The gall-bladder is distended; the gall-duct patulous. On section the liver presents a typical nutmeg appearance, and the substance of the organ is apparently not at any point invaded by the gelatinous material. On opening the thoracic cavity the lungs extend well forward; pericardial sac contains a small amount of clear, straw-colored fluid. On opening the pericardium the heart is somewhat small, the heart muscle of a pale, brownish color. In the right ventricle is a large rose-colored clot, which extends into the pulmonary artery. The lungs contain a normal amount of air, show grayish and red mottling, but no evidence of metastases. Projecting into the left pulmonary artery is a large thrombus, adherent at various points and extending into the branches of the pulmonary artery. The bronchi contain frothy exudate. The right lung presents the same characteristics as the left, and the right pulmonary artery likewise contains a thrombus. The transverse and descending aorta shows a moderate amount of sclerotic change. The bladder and genito-urinary tract are free from abnormalities. The omentum and the cystic mass attached to the cæcum were removed and preserved for further examination.

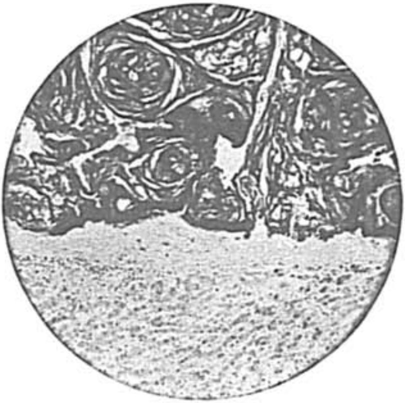
Anatomical Diagnosis. Colloid carcinoma of the peritoneum; thromboses of the pulmonary arteries; atheroma of the aorta; brown atrophy of the heart muscle.

The microscopical examination of the fresh material from the vesicles of the tumor shows it to consist of a finely granular or homogeneous material, containing a considerable number of degenerated epithelial cells and the small spherical bodies noted in the peritoneal fluid. Examination of the peritoneal fluid from the cadaver showed that it likewise contained large numbers of the various forms of the organism. Portions removed from different parts of the tumor were hardened in various hardening reagents. The examination of the hardened and stained sections shows the tumor to be an adenocarcinoma of the omentum, which has undergone advanced colloid degeneration. The gelatinous vesicles show the typical appearance and characteristics of colloid carcinoma. They penetrate between the muscle fibres of the diaphragm into the layers of the capsule of the liver and involve the

PLATE I.



1.
Adenocarcinoma. Case I. (M. P.) (H. E.)



. 2.
Colloid mass from under surface of liver. Case I. (M P.) (Methylene blue.)

entire thickness of the omentum. The muscle fibres or connective tissue structures are forced apart by the gelatinous material.

In the neighborhood of the cæcum the colloid degeneration of the intestine is not so marked as in the other portions. Here the epithelial structures may be seen invading the abdominal wall and the mass of adherent structures which surround the growth. The description of a section from the growth in the neighborhood of the cæcum and one from the colloid portions of the tumor will include the principal characteristic features.

Slide 2. A section taken from the region of the primary tumor, hardened in alcohol, stained with Delafield's hæmatoxylin, includes a portion of the subperitoneal fat and the wall of the tumor. The connective tissue stroma of the section is thickly infiltrated with round cells. In the deeper portions of the section are a number of muscle fibres, likewise infiltrated with round cells. Extending through the entire thickness of the section are large, epithelial, glandular structures and nests of isolated cylindrical epithelium. (Plate I., Fig. 1.) Near the surface of the tumor these glands are broken up, and the epithelium is scattered through the stroma without definite arrangement. The cylindrical form of the epithelium is likewise changed, and here and there concentric structures closely resembling epithelial pearls may be noted. In the large glandular structures the epithelium is commonly several layers deep. The nuclei are large, deeply-stained, of irregular form and irregularly placed. The protoplasm of the cells stains deeply. Karyokinetic figures are uncommon. When examined under high power a number of the cells are found to contain typical Plimmer inclusions and the various forms beginning with small spherical bodies known as Russell fuchsin bodies and certain deeply stained bodies within the nuclei and protoplasm of the cells, which we now recognize as the younger forms of the parasite.

A portion of the tumor covering the under surface of the diaphragm, the surface of the liver and the peritoneal surface, which shows the typical vesicle formation characterizing this growth, reveals, on microscopical examination, the typical appearances and structure of an adenocarcinoma in an advanced stage of mucoid degeneration. The description of a single slide will suffice.

Slide 10, from material hardened in Flemming's mixture, embedded in paraffin and stained with iron hæmatoxylin Bordeaux red, through a large mass of gelatinous material from the under surface of the diaphragm. Tumor is divided into large vesicular structures, which, under the microscope, are divided up into smaller vesicles. These are filled with coagulated material, in which are embedded a number of epithelial cells in various stages of mucoid degeneration. The nuclei of these cells are somewhat smaller than those seen in Slide 1. In some of the vesicles the cells are of a typical cylindrical type, arranged in rows in the usual manner found in adenocarcinoma. Nearly every cell contains a large vesicle apparently filled with mucoid material. In some of the vesicles the cells are scarcely discernible, only occasional chromatin granules and indefinite masses of protoplasm marking the site where the cells once existed. The stroma is thin, contains but few nuclei, and occasionally, at the intersections, a few fat cells and a well-defined round-celled infiltration. The nucleus of nearly every cell contains a deeply stained body (nuclear infection). The occasional vacuoles filled with mucoid material present an entirely different appearance from possible Plimmer inclusions.

Slide 12, taken from a mucoid portion of the tumor, near the site of the original tumor. Material hardened in sublimate, stained with methylene-blue. The tumor is made up of large and smaller vesicles filled with mucoid material. Within the vesicles may be seen the remnants of adenomatous structures, the epithelium of which shows marked mucoid degeneration. Under high power the cells of this portion of the tumor are found to closely resemble the adenomatous structures seen in Slide 2.

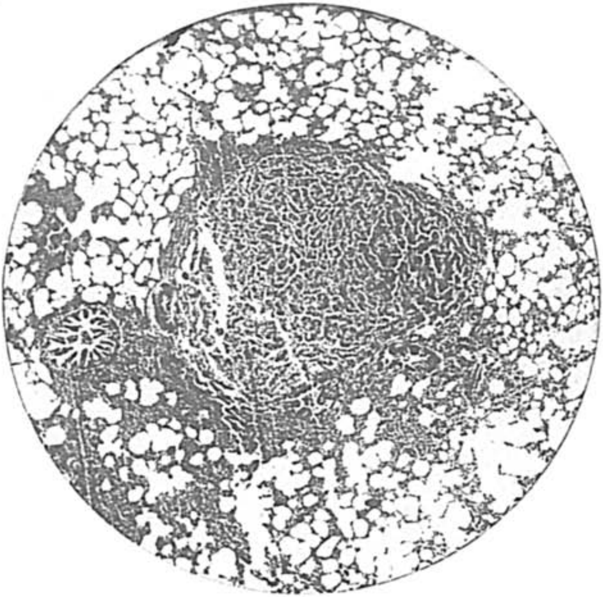
Slide 13. Section of a large mucoid mass removed from the under surface of the liver, stained with methylene-blue. The vesicles of this section contain deeply-stained masses of mucoid material. These are sharply delimited against the underlying structures. Embedded in the material are the fragments of degenerated epithelium. No well preserved epithelial elements are present. (Plate I., Fig. 2.)

Cultures made from different portions of the tumor were all bacteriologically negative. The usual forms of bacteriological culture media were employed. These were placed under aërobic and anaërobic conditions.

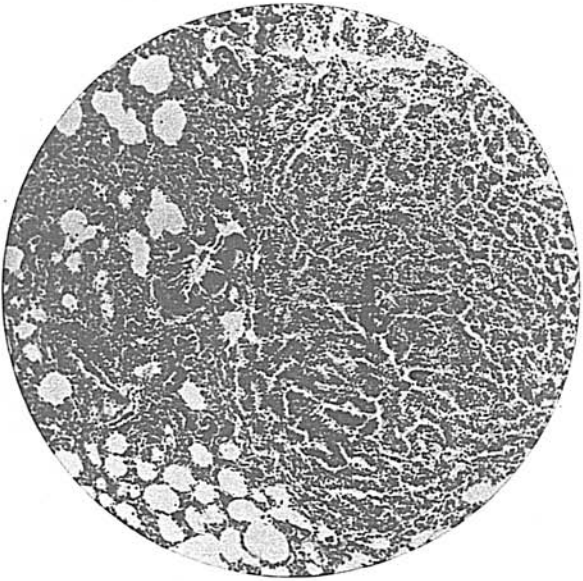
On September 6th the following note appears in the *Laboratory Journal:* " Guinea-pig No. 1 shows distinct evidence of listlessness and does not appear well. The animal sits in the corner of its cage and does not move about." On Wednesday, October 14th, both Pigs 1 and 2 were killed.

The following notes taken from the autopsy of Pig 1 will be of interest:

Inspection of the site where the injection of the serum was made in the right jugular shows that the superficial wound has healed, and at the point of ligation the jugular vein is transformed into a fibrous cord. On opening the thoracic cavity nothing of interest is to be noted until the lungs are reached. These are found to contain a normal amount of air. The pleura appears slightly injected. On opening the pleural cavity cultures are made from the lung surface. The lungs contain a large amount of pigment and show areas of venous engorgement with occasional minute hemorrhages beneath the pleura. After removing the lungs and incising them, both are found to contain a large number of minute nodules varying from the size of a pinhead to one larger than the rest, about the size of a grain of rice. These nodules are white, of greater consistence than the lung tissue, and somewhat elevated above the cut surface. Careful examination shows them to be located near or surrounding the small bronchi. The small areas of hemorrhage noted on opening the thoracic cavity are found to be confined to the region immediately beneath the pleura. On examining the abdominal organs the stomach is found to be empty, the intestines collapsed, the bladder widely distended with urine, and the cortex of each kidney of a deep red color. The capsules strip with some difficulty, and the stellate vessels directly beneath the capsule are slightly injected. The spleen is somewhat enlarged, shows a mottled, bright-red, and reddish-brown appearance. On incision the follicles are unusually large and stand out plainly against a background of deep red and reddish-brown pulp. Fresh microscopical examination of the heart's blood shows it to contain,



Section of Lung, Pig I, including the largest nodule. Primary Adenocarcinoma following injection of protozoa in peritoneal fluid of Case I. (Low Power.)
(Hæmatoxylin Eosin.)



Margin of nodule (Plate II) showing origin of tumor from epithelium of bronchus. (Middle Power.)

beside the normal constituents of the blood, a large number of small, spherical, highly refractive bodies closely resembling fat, which in size and appearance are identical with those found in the original fluid with which the animal was injected. Aside from the minute white nodules in the lung, the pigmentation and enlargement of the follicles of the spleen and lymph nodes, and the injection of the kidneys, the remaining organs of the animal show no abnormalities. The lungs were hardened in formalin.

A large number of sections were cut, some of which include the largest nodule above mentioned. On examination with low power of a section stained with Delafield's hæmatoxylin, the nodule is readily found by the deep staining of the structures which compose it. On closer scrutiny, even with low power (Plate II.), the structure presents the appearance of a rapidly-growing nodule possessing a definite structure. The lung trabeculæ in the immediate neighborhood, especially in a portion adjacent to a bronchus, shows marked interstitial thickening. In the immediate neighborhood is a section of a middle-sized pulmonary vessel and a good-sized bronchus. The remains of a collapsed bronchial artery are seen just at the periphery of the growth. The alveoli of the lung show marked concentric flattening about the periphery of the nodule. A few yellowish-brown spots indicate the presence of pigment within the growth. Under high power the structure of the nodule becomes distinctly apparent. (Plate III.) It is made up of epithelial cells arranged in a characteristic manner upon a delicate connective tissue stroma. This forms the axes of longer and shorter papillary projections, which are closely packed together. Upon these are arranged single and double rows of cylindrical epithelium. At the periphery of the growth the alveoli of the lung are concentrically flattened, and the structure of the adenoma merges gradually into the structure of the lung trabeculæ. In one or two points the adenoma is spreading into the surrounding tissues, and in these localities the trabeculæ of the lung show a marked thickening and a distinct round-celled infiltration.

Under still higher power the nature of the epithelial cells may be more distinctly observed. They are fairly uniform in size, the cell protoplasm is abundant and palely stained. The nuclei are oval, are placed near the base of the cells, and stain deeply. The cells are cylindrical in form, but at the periphery, where the compression is great, they show evidence of considerable distortion. Included within the tumor are a number of cells containing coal pigment, and in the stroma are a few capillaries containing blood cells. In the neighborhood of these capillaries are occasional connective tissue cells loaded with hæmatogenous pigment.

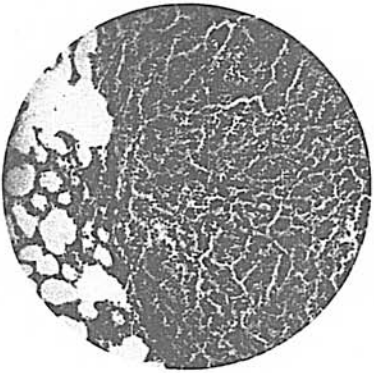
The bronchial vessel lying directly at the margin of the growth is collapsed; the intima shows proliferative changes; the media is somewhat thickened. The adjacent pulmonary vessel is surrounded by a thick layer of cells, which apparently spring from the adventitia of the vessel. These are of the typical round-celled variety, and in certain portions are accumulated in groups, forming well-defined nodules. Careful scrutiny of the surrounding pulmonary tissue, especially in that portion in which there is great thickening of the trabeculæ, shows this change to be due to a proliferation of the connective tissue stroma of the lung. A large number of cells closely resembling the *epithelial* *cells of the lung are mixed with these.* A certain number of the last-mentioned elements contain deposits of coal-dust. An examination of the other pulmonary vessels of the lung reveals the same proliferation of the adventitia, and many of the smaller vessels are surrounded by nodes or rings of closely packed round cells. (Plate IV., Fig. 2.) In various other portions of the lung are areas in which the trabeculæ are thickened or the alveoli completely obliterated. Within these areas will be found nests of epithelium, and in the larger, well-defined adenomatous structures identical in appearance with that of the largest nodule. (Plate IV., Fig. 1.)

Since the acquisition of Plimmer's method we have restained sections of this case, and find that every epithelial cell in the nodule contains a deeply stained nuclear body, which we now recognize as young parasites. The lung trabeculæ are infiltrated with large numbers of young organisms, which can only be recognized from round cells by their form and homogeneous nature. They are either round or oval, or appear to have been hardened in the act of projecting pseudopodia. In some cases they are closely packed together, and a considerable number are found in the bloodvessels. By carefully investigating the different parts of the lung, it will be found that the epithelial infection, although present wherever nests of epithelium are formed, is not uniform, and that certain bronchi and areas of the lung have escaped. By such a comparison we may rule out the possibility of these bodies being due to a peculiarity of the staining method, and a careful comparison of a large number of sections shows that the presence of these bodies in certain localities, especially in the carcinomatous nodule, holds for all the sections made, although the staining method may have been slightly modified. Directly beneath the pleural surface are areas in which the alveoli are filled with blood and the capillaries are markedly engorged. These areas correspond to the small hemorrhages observed macroscopically.

Examination of sections of the spleen shows it to contain a large amount of brown pigment deposited in the pulp. Evidence of widespread hemorrhage is also found, and the follicles are markedly enlarged. (Plate V., Fig. 1.) In sections stained with Plimmer's method the nuclei of a large number of the cells of the follicles contain a deeply stained central body—in some cases spherical, in other cases of irregular shape. These we believe are young parasites. (Plate VI., Fig. 2.)

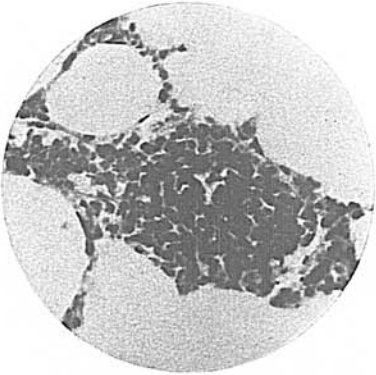
The pigment in the pulp takes the form of triangular and irregularly shaped plates, and is very abundant. The pulp contains a large number of lymphocytes and red blood-corpuscles. Scattered between the cells in the pulp are a large number of extracellular parasitic bodies, which conform in size to the pale forms which in the fresh state are capable of sending out pseudopodia. (Plate V., Fig. 2.) (Plimmer's stain.)

Summary of Case I.: The facts in this case may be summarized as follows: The primary growth was adenocarcinoma, probably having its origin in the appendix. This had spread, involving the greater portion of the peritoneal surface, with infiltration of the omentum and mesentery. The greater portion of the tumor had undergone mucoid degeneration, and the peritoneal cavity was filled with clear, straw-colored fluid. The patient was opened aseptically. A test-tube of this fluid, which was



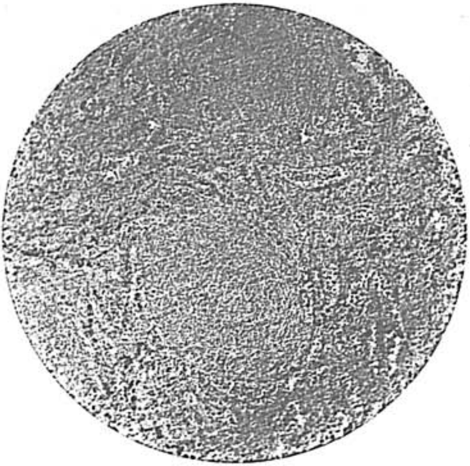
1.

Margin of smaller nodule, Pig I. (M. P.) (H. E.)



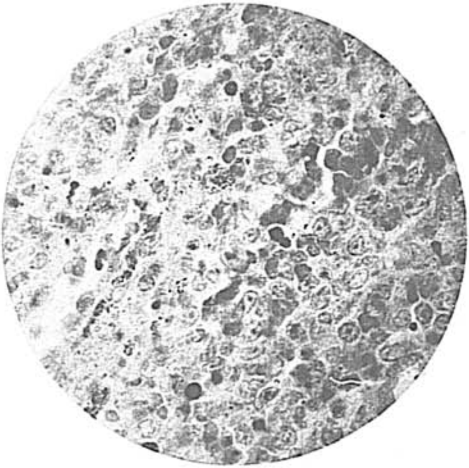
2.

Typical focus of round cells in perivascular lymph space of small vessel.
Lung Pig I. (H. P.) (H. E.)



1.

Section of spleen, Pig I, showing enlarged follicles and hæmatogenous pigment deposited in pulp. (L. P.) (H. E.)



2.

Periphery of follicle of spleen, Pig I, free Russell's bodies (young protozoa) between the cells. (H. P.) (Plimmer's method.)

removed through a sterile tube, and which remained bacteriologically sterile after an incubation period of thirteen days, contained a large number of small hyaline bodies, which were observed under the microscope to increase in size and change their form and pass through a cycle of development to what appeared to be a spore-forming stage, was injected into three animals—two, a dog and a guinea-pig, in the peritoneal cavity; one, a guinea-pig, in the jugular. The guinea-pig and dog which were inoculated in the peritoneal cavity developed no tumor formation, but a marked peritonitis and enlargement of the regional lymph nodes. The abdominal cavities of each animal contained a small amount of fluid; and in this fluid we were able to detect the characteristic spherical bodies, nucleated bodies, and sacs filled with the granules already described as being present in the primary case.

Slides taken from this fluid and incubated for a period of three or four days in the thermostat showed that the bodies not only changed their form, but we were able to trace the development of the larger structures from the small hyaline forms. Of particular interest was a portion of a slide so treated, in which we found numbers of the organisms which had sent out pseudopodia toward a neighboring air-bell. There were at least twenty organisms so arranged, and all of them had run out long pseudopodial projections to the margin of the air-bubble.

The animal injected in the jugular was killed after fifty days. On section the lungs were found to contain minute white nodules, which, on microscopical examination, proved to be beginning foci of adenocarcinoma. The pulmonary bloodvessels showed marked proliferation of the epithelial and adventitial cells. The spleen of the animal contained a large amount of blood-pigment, and the Malpighian corpuscles were enlarged. The cells of the tumor and those of the enlarged follicles in the spleen were each found to contain within the nucleus irregularly-shaped, deeply-stained bodies of unusual appearance. About the periphery of the follicles and scattered through the splenic pulp were a number of round and oval bodies, which corresponded morphologically to what are known as Russell's fuchsin bodies. In the perivascular lymph spaces of the lung we have since been able to detect, by employing Plimmer's staining method, the presence of the half-grown organisms in large numbers, which corresponded to those described by him as of constant occurrence in carcinoma in man.

Our attempts to cultivate these organisms at the time of this experiment were not followed by success, as the forms of culture media employed were unsuitable. As will be seen later, in the experiments which shortly followed upon the one just described we met with better success, and succeeded in cultivating with comparative regularity, directly from cancer, from fluids which were in contact with cancer, and from experimental animals, the organisms which have been described in this first experiment. The medium which has thus far given us the best results is that recommended by Celli for the cultivation of amœbæ, fucus crispus bouillon.

Two other animals were inoculated at this time—one, a guinea-pig, in the peritoneal cavity, which was killed after nineteen days, and a dog which rapidly became emaciated and died after sixty-four days, presenting marked evidence of peritonitis with general enlargement of the regional lymph nodes, enlargement of the spleen, and œdema of the lungs. In the peritoneal fluid and the heart's blood, as well as from the organs of these animals, we were able to detect large numbers of the parasites. Sections of these organs, which have since been stained with Plimmer's method, reveal the presence of large numbers of the parasites in all of the viscera, however, especially numerous in the lymph nodes and lungs.

A number of tubes of the serum from this first case have been retained in the laboratory, and animals have been inoculated with them at later periods. Of special interest are Guinea-pigs 40, 42, and 43, which were inoculated with this serum which had stood for four months, with no other precautions being taken than placing the tubes which contained it in a cool place. The animals inoculated at this later period gave identically the same results as those of the original experiments.

During the period of these first experiments I was fortunate in having as an assistant Dr. F. C. Busch, who now occupies the chair of physiology in the University of Buffalo. Dr. Busch was of the greatest service to me, and assisted me in all of the work of that period with great skill and devotion.

Following these first experiments our efforts were especially directed toward an investigation of the fresh scrapings of cancers, and in attempting to demonstrate these bodies in the tissue in hardened sections. Having once established the fact that the small spherical bodies which so closely resemble fat were not fat (giving no reaction with osmic acid[1] and not being affected by fat solvents) we set about to determine how the great discrepancy between the large number of parasites found in fresh cancer and in sections could be accounted for.

A careful examination of a large number of tumors, including those removed by operation, shows that in all rapidly growing tumors, especially when of large size, a great number of organisms are present. Small tumors, as a rule, were found to contain only the smaller forms of the organisms; the tumors and organs removed from cadavers of patients who had died from carcinoma or sarcoma showed the presence, especially in the tumors, of great numbers of the organisms in all phases of development. After comparing tumors removed by operation and those from cadavers it becomes evident that the organisms either increase very rapidly during the period just before death or that they proliferate in the tissues after death. In two cases of large-sized tumors, which immediately after operation contained a predominating number of the small forms of the organism, and which were retained sterile, we were able to make the following observations:

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[1] In determining the relation of these bodies to osmic acid, fat was used as a control and placed under the same conditions. In one or two cases, after a treatment of one or two days with osmic acid, the writer has observed a slight browning of the periphery of these bodies, but fat used as a control had long before given the intense black reaction which characterizes it. We are not certain whether or not it is possible to cause a browning or possibly even blackening of these bodies by osmic acid if treated in some special manner. This question will be the subject of a special piece of research in the laboratory, but will in no way affect the significance of our findings. Sections hardened in Hermann's fluid in which the fat in the tissue gives the usual black reaction when cleared in origanum oil and covered with a cover-glass without staining show bodies that are of a yellowish color.

An examination of successive scrapings from the tumor, several hours apart, in each case showed that the relative size of the organisms gradually increased. In the course of ten hours repeated scrapings showed that the amœboid forms were greatly increased in number, and after twenty-four hours the spore sacs of the organism were present, for the first time, in large numbers. Continuing our observation up to a period of about three days, we observed in these two cases that the sacs were ultimately replaced by groups of hyaline bodies, which were considerably larger than those which the sacs originally contained. It will be seen from this observation that the so-called fatty degeneration of carcinoma is at least in some part due to the presence of the various forms of the organism which have been mistaken for fat droplets and infected epithelial cells which were supposed to be in an advanced stage of fatty degeneration.

We were likewise able to determine that in the centre of carcinomata which had undergone degeneration the fluid, the so-called cancer milk of the older writers, consists practically of a pure culture of these organisms. The fluid from malignant ovarian cysts likewise contains a large number of the organisms, and the peculiarly characteristic mush found in the cavities of certain adenocarcinomata of the ovaries is likewise largely composed of the various forms of the parasite.

We were thus forced to conclude that bodies identical in appearance to those observed by us in the peritoneal fluid of our first case could be found in all scrapings of cancer. The small, highly refractive form which in suspension possesses a characteristic oscillating motion, the larger pale forms projecting pseudopodia, and the saccular forms containing highly refractive spherical bodies could be detected with equal facility in the fresh scrapings of any malignant tumor. The small form of the organism which so closely resembles fat, and the larger spherical forms containing fine granules are particularly abundant. By incubating hanging-drop preparations of fresh scrapings from cancer the smaller forms can be followed in their development, during which they grow in size and finally become granulated, and, if kept upon a warm stage, ultimately throw out pseudopodia, develop a nucleus, and end by turning into a sac containing the spores of the organism. Owing to the fact that the specific gravity of the organism is less than water, it rises to the surface, and must be sought directly beneath the cover-slip and not in the lower portions of the fluid. This fact we had noticed and made use of before the recent publication of Funk on the vaccine organism.

Having ascertained that a large number of organisms were invariably present in cancer, we undertook to determine why these organisms could not be demonstrated with the ordinary staining methods in the tissues, and were able, first of all, to determine that the application of almost all fixatives caused the disappearance of all the spore sacs of the organisms, and the greater part of the large spherical and granular bodies. Only the small, more resistant forms of the organism remained, and these we were able to stain in a large number of sections with the aniline dyes, in which case they presented the form which has already been recognized and first described by Russell, known as "Russell's fuchsin bodies." The larger forms which are still hyaline in character or contain fine granules, and which might be spoken of as the quarter-grown organism, in sections stained by the ordinary methods so closely resembled free nuclei and round cells that it was impossible to state which were parasites and which tissue elements. In one case, however, of carcinoma of the bladder, observed during this period, where the organism appeared in large numbers in the urine, we were able to detect, even in hæmatoxylin preparations, the quarter-grown form of the parasite between the epithelial cells, and attached to the surface of the tumor after removal.

In the summer of 1898 the writer was asked to make an autopsy on a female patient who had died of carcinoma of the uterus. On opening the peritoneal cavity we were surprised to find an advanced general peritonitis. There was considerable clear fluid in the peritoneal cavity, and the surface of the peritoneum had lost its brilliancy. The intestines were matted together, and the thought was immediately awakened that a perforation of the vagina had probably occurred as the cancer involved the cervix, and that bacteria had entered the peritoneal cavity and had thus produced a secondary infection. On examining the spleen it was found that nearly one-half of that organ was in a condition of infarction; and on examining the lungs they were found to be markedly œdematous. Cultures for bacteria were made from the peritoneal cavity, the spleen, the blood, and the lungs, with the result which was determined later that they all remained bacteriologically sterile.

On returning to an investigation of the pelvic organs, we were immediately struck with the fact that there was no perforation, which at once directed our attention to the possibility that the case might be one of general infection with the organism of cancer. For this reason we immediately made fresh preparations from the surface of the peritoneum, the substance of the spleen, the lungs, the heart's blood, and one of the larger superficial veins of the lower extremity. In all of these were found large numbers of the pale hyaline forms which we had already learned to recognize as constant in scrapings from carcinoma, in the peritoneal fluid of carcinomatous patients, and in the heart's blood and peritoneal fluid of our experimental animals. Dr. Irving Phillips Lyon, associated with the laboratory, was present with me at the autopsy, and confirmed my observations.

This was the status of our work in December, 1898, and it was upon the basis of these facts that the writer made the statement before the Medical Society of the State of New York, in January, 1899, which was quoted at the beginning of this paper. It will be seen from this statement that we believed we had recognized and demonstrated the presence of parasites in cancer and had successfully produced cancer in one animal, but that we were not at that time in a position to state what the nature of these parasites might be.

During the winter of 1898–99 the writer planned an elaborate series of experiments, based upon our original experiment, which are only just completed. With the clew afforded by the findings of the autopsy described above we have examined since that time the organs of a number of cadavers which have died from cancer, and on the bases of these observations we are prepared to state that *all the organs, including the blood taken from all regions of all cases dying of cancer, including sarcoma and epithelioma, contain large numbers of the organisms.*

Following the same lines, we have likewise observed *in all cases of carcinoma and sarcoma thus far examined in which cachexia was well marked, that the organisms, especially the younger forms, can be detected in the peripheral blood.* In these cases the smaller forms of the organism possess the peculiar oscillating motion already described. The quarter-grown forms of the organism are usually actively sending out pseudopodia, and conform very closely in appearance to the amœboid bodies found in the blood by Pfeiffer and Reed after vaccination and in cases of smallpox. (The question of the time of appearance of these organisms and the utilization of this fact as a means of diagnosis already forms the subject of a piece of research in the laboratory.) Animals may be infected by inoculating them with the peripheral blood from cachectic cancer cases when the serum contains the organism. In these cases the organism possesses great virulence. The younger forms of the organism may likewise be found in the peripheral blood of the animals after inoculation with cultures or carcinomatous material. Figs. 2 and 3, Plate VI., represent the larger hyaline forms of the organism from the blood of a case of fatal malignant lymphoma obtained seven days before death.

Having reported before the State Society our findings of parasites in cancer, it remained for us to determine the nature of these organisms and to continue our investigation in the most comprehensive manner possible. It became at once apparent that there could be but two possibilities in the case—either that the organisms were of an animal nature, and were then protozoa, or that they belonged in the vegetable kingdom, in which case they were in all probability low forms of fungi, probably yeasts. In order to understand the difficulties which prevented our arriving at an earlier conclusion as to the nature of these parasites it is well to review the status of scientific research at that time.

When, in 1898, the State of New York established a laboratory for the investigation of cancer, the attention of the scientific world was attracted by a series of five articles published by an Italian investigator, Sanfelice, of Cagliari, Sardinia, all of which appeared in the *Zeitschrift für Hygiene und Infectionskrankheiten.* Beside these, a number of articles appeared in different periodicals by a second Italian investigator, Roncali. These last are of less importance. The purport of the articles of both of these investigators was that the parasitic inclusions found in cancer were identical in appearance with yeast organisms or blastomycetes when injected into the tissues of animals. Sanfelice's articles went further, and in the twenty-ninth volume, 1898, of the above-mentioned journal, he published an account of tumors which he succeeded in producing by inoculation with a yeast in two dogs, both of which he wishes to designate as true adenocarcinoma forming metastases.

Before entering into a discussion of the merits of Sanfelice's investigations it is well to state that the first observer to interpret the spherical, hyaline bodies found in cancer as blastomycetes was Russell. Russell published an article in the *British Medical Journal*,[1] in 1890, in which he claimed that he had detected a micro-organism to which he gave the non-committal name of "fuchsin body." These were found in groups or clusters of from two or three to twenty or more. They were from four to twelve μ in diameter, were apparently homogeneous, and in some cases the groups of bodies were held together by a faintly stained material. He believed they belonged in the yeast group (Sprosspilze). Russell made no attempts to cultivate these organisms. His publication

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LEGEND OF PLATE VI.

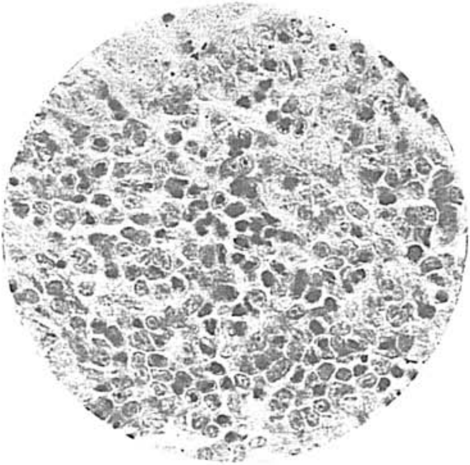
Fig. 1. Centre of follicle of spleen, Pig 1. A large number of cells contain deeply stained bodies within the nuclei (internuclear infection).

Figs. 2 and 3. Young forms of parasite from blood of case of malignant lymphoma. (Oil immersion, fresh preparation.)

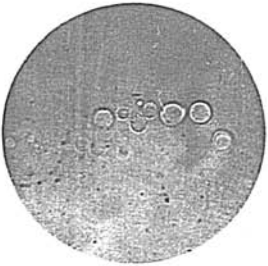
Fig. 4. Very small and quarter-grown form of parasite from case of carcinoma of pylorus involving omentum. Culture obtained from fragment of metastatic deposit on fucus crispus bouillon. (Oil immersion, fresh preparation.)

[1] An address on a Characteristic Organism of Cancer, December 13, 1890, vol. ii., p. 1356.

PLATE VI.



1.



2.

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4.

led to very heated discussion, and his work was attacked, especially by Shattuck and Ballance, *British Medical Journal*, 1890, vol. i., p. 565, and Klein, *Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie*, 1891, Bd. xi. The first of these authors described similar bodies found in caseating lymph nodes and the walls of senile arteries, while Klein was of the opinion that Russell's bodies bore a close relation to Altmann's cell granulæ.

In 1895 Busse, a German investigator, published the first description of an undoubted yeast, which he found to be the cause of a fatal infection in man. This organism he succeeded in isolating in pure culture, and found that the lesions which it produced were a combination of abscess and tumor formation ("Ueber Saccharomycosis Hominis," *Virchow's Archiv*, vol. cxl., p. 23). The appearance of the organisms in the tissue was so suggestive of the cell inclusions in carcinoma that Busse had previously published a description of the case under the title "On Parasitic Cell Inclusions and their Cultivation," in the *Centralblatt für Bacteriologie*, vol. xvi. The organisms were very commonly within the large cells which composed the walls of the abscess cavities, and under these conditions presented an appearance very much like the cell inclusions of carcinoma. In 1897 Busse published a monograph entitled *The Yeast Organism as the Cause of Disease*. In his last publication he is distinctly of the opinion that the pathogenic yeasts and the cell inclusions in cancer have no relation to each other. He describes a series of experiments which he carried out in the hope of causing proliferation of the cell inclusions of cancer. For this purpose he planted small portions of tumor in which he had detected the inclusions in various kinds of culture media, and placed them for several days in a thermostat. In none of these experiments was he able either to obtain a culture of organisms or to observe that the cell inclusion showed any evidence of increase in number or change of form. He likewise attempted to inoculate animals with small portions of lymphosarcoma, with negative results. He included a short description of all of the organisms of the same class described up to the time of his publication, and because of the negative results obtained and the fact that no positive evidence had been produced to show the identity of cell inclusions and yeast organisms, he concluded that they had nothing to do with each other, and that the yeast organism played no rôle in the production of carcinoma. Beside Busse, a number of observers have since described lesions of various kinds (especially skin lesions) produced by yeasts.[1]

The articles of Sanfelice are of the greatest interest, as he has undertaken to elucidate the subject by a careful study of wild yeasts and such as he found to possess pathogenic properties for animals. Among these was one obtained by cultivation from the skin of a lemon, which, when injected into animals, produced granulomata. These, in his opinion, closely resembled sarcoma, for which reason he gave the organism the name "saccharomyces neoformans." In 1898 Sanfelice published an account in which he stated that in his experiments he had inoculated, up to 1896, fifty-nine dogs. His method of inoculation he had varied as much as possible—some in the subcutaneous connective tissue, others in the testes, others in the breast, the spleen, the liver, the lungs, the jugular vein, and the peritoneum. Most of the animals showed no pathological change and were killed after three, four, and five months. In the greater number of these animals Sanfelice was unable to cultivate the organisms from the region of the inoculation, but in most cases he was able to secure them from the regional lymph nodes. By transferring his organism from dog to dog he succeeded in increasing its virulence to such an extent that, while in the beginning when injected into the jugular vein it produced no lesions, in the end similar inoculations resulted in the death of the animal, with the development of characteristic changes in various organs. Beside simple inoculations, he practised various methods to reduce the resistance of the animals, such as drawing a portion of their blood, injuring the tissues before inoculation, and introducing foreign bodies at the time of the inoculation. None of these devices produced any further results than those obtained by the simple inoculation. He ultimately succeeded in obtaining what he described as a positive result by inoculating a female dog with a culture of saccharomyces neoformans which had passed through a large number of dogs. A small portion of the culture was removed with a platinum needle, emulsified with sterile water, and injected into the breast with an ordinary sterile syringe. After the first few days the breast showed a certain amount of swelling and inflammatory reaction. *This disappeared after a few days and the breast presented a normal appearance.* After several months he observed that the breast in the neighborhood of the inoculation began to swell, and this increased gradually until a definite tumor was formed, which at the time of the death of the dog, ten months later, was about half the size of an egg. With the development of the tumor the dog showed definite signs of emaciation. The tumor was of greater consistence than the breast, and the nipple was retracted. The skin in one or two places was apparently adherent to the tumor. The inguinal lymph nodes on both sides were somewhat enlarged, the largest about the size of an almond, the smallest the size of a hazel-nut. The organs of the thorax and head showed no pathological changes.

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[1] Gilchrist: Johns Hopkins Hospital Reports, vol. i. Gilchrist and Stokes: Journal of Experimental Medicine, 1898. L. Hektoen: Ibid., vol. iv., p. 261.

Microscopical sections made through the tumor showed that the centre of the structure did not possess the same histological characteristics as the periphery. At the centre the tumor presented the appearance of the normal breast of the dog. At the periphery the histological characteristics of the tumor were such, to judge from the description of the author, as to strongly suggest malignant change. Instead of the single layer of epithelium lining the spaces in the connective tissue stroma, which characterized the centre of the tumor, the portions near the periphery were lined by multiple layers of large epithelial cells. The nuclei were irregularly arranged, of varying size and form, and the membrana propria of the canals was missing. Isolated nests of epithelium were likewise to be found in the stroma. The epithelium exceeded in amount the connective tissue stroma. Sanfelice found that the enlarged lymph nodes on section presented the appearance of containing metastatic deposits of the large tumor. Sections cut from these lymph nodes showed that they actually contained epithelial structures of similar form and appearance to the peripheral portions of the tumor.

From the description of the tumor given, indicating the development of metastases in the regional lymph nodes, if the description of the author can be taken, there is little reason to suppose that the case was not one of true adenocarcinoma. *From the original tumor and all of the lymph nodes, Sanfelice was unable to obtain cultures of his organism, and neither in the metastatic deposits nor in the primary tumor was he able to demonstrate bodies which were unquestionably yeasts.*

Beside the case just mentioned, Sanfelice describes a second dog, which was inoculated in the testicles, and which, after a period of four months, had developed a well-defined enlargement of the organ and several nodular masses, which appeared to be enlarged regional nodes. Several nodular masses were found within the prepuce of the animal, and Sanfelice observed that from the orifice a small amount of purulent fluid could be expressed, in the cells of which he detected inclusions closely resembling the bodies of Russell, which he considered to be altered yeasts.

The animal died unexpectedly during the sixth month, and at the autopsy, which was performed with the assistance of Prof. Charbone (pathologist), the nodular enlargement of the testicle was found to be of yellowish-white appearance, of definite consistence, which could not be sharply differentiated from the tissue of the testicle. On both sides of the penis bone were a large number of apparently metastatic deposits —one of these as large as a hazel-nut, the majority about the size of peas. Surrounding the glands was a conical mass of newly-formed tissue, which communicated with the preputial opening. The primary tumor appeared to be in the testicle, and the smaller deposits appeared to be of a metastatic nature. The inguinal lymph nodes were slightly enlarged, and on section presented a normal appearance. The spleen, kidneys, and liver were slightly hyperæmic, but showed no other changes.

As the autopsy revealed no well-defined cause for the exodus, the decision was reached that the animal had been surreptitiously poisoned. Cultures which were made by emulsifying portions of the tumor and metastases were all negative. Portions of the tumor were used to inoculate other animals. At the time of the publication Sanfelice was not in a position to state the outcome of these experiments. Sections of the tumor showed it to be very probably of epithelial nature. The cells of the tumor were rather small, and closely resembled those of the basal cells of the seminal vesicles of the dog.

In 1898 the writer had the pleasure of making Prof. Sanfelice's acquaintance and carefully going over with him the sections from these two cases and obtaining from him a culture of the saccharomyces neoformans and blocks of tissue from the two tumors. It is important in estimating the work of an observer to know something of his character and temperament, and for that reason it was a great pleasure to make Prof. Sanfelice's acquaintance. He is a man of unquestionable sincerity and honor, and no matter what the interpretation of the work which he has published may be, his statements can be accepted as accurate so far as observation can make them. There is absolutely no question that the tumor of the breast with metastases in the regional lymph nodes, described by Sanfelice, would pass muster as an adenocarcinoma.[1]

The courtesy which Prof. Sanfelice showed me in placing before me and at my disposal all of his material has been of the greatest service to the laboratory, and I wish to take this occasion to publicly thank him for his courtesies. The culture which he forwarded to us was that of an ordinary yeast, so far as we are able to classify it. The appearance of the culture and of the fresh organism is shown in Plate VII., Figs. 1 and 2.

The publications of Roncali have been confined to the description of attempts to cultivate yeasts from carcinomata. He describes several successful attempts in which he employed acid sugar-water as the medium. The writer was not deeply impressed with the preparations which Prof. Roncali showed him, and, as his cultivation experiments are so widely at variance with those of other observers and ourselves, we feel that they do not require a detailed description.

In the April number of the *Practitioner* for 1899 there appeared an article by Plimmer which is without doubt the most important communication in a number of that journal devoted entirely to the subject of cancer. After a short review of the literature, and an analytical discussion

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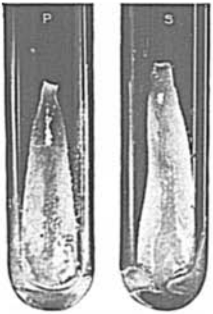
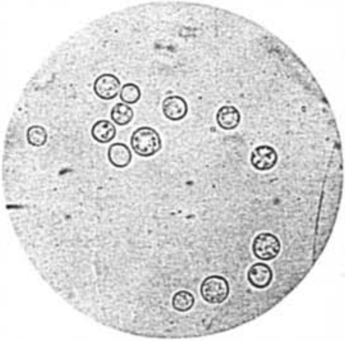
[1] The probable significance of these experiments will be considered in Part II. of this article.

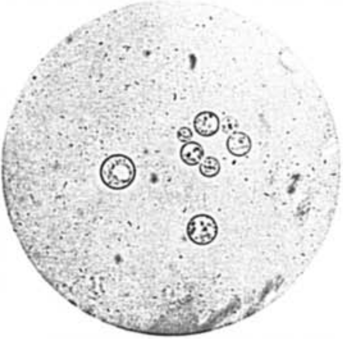
PLATE VII.



1.
Potato cultures of Plimmer's yeast and Sanfelice's "Saccharomyces Neoformans."



2.
Fresh preparation of "Saccharomyces Neoformans," Sanfelice. (Oil immersion.)



3.
Fresh preparation of Plimmer's yeast. (Oil immersion.)

of the structure of cancer, Plimmer gives the method by which he has examined a large number of cancers of various types. He recommends various hardening and staining methods for the demonstration of cellular inclusions, and, as in our experience one of these methods gives pre-eminent results, it would seem desirable to introduce it at this point:

1. Small slices of tissue are hardened in Hermann's fluid twelve to twenty-four hours.
2. Thorough washing in running water, twelve to twenty-four hours.
3. Harden in alcohol; embed in paraffin.
4. Remove paraffin in xylol.
5. Absolute alcohol.
6. Place in peroxide of hydrogen until the black is removed from the section and no further bubbles form upon the surface, one-quarter to half an hour.
7. Wash in water.
8. Stain with Haidenhain's iron hæmatoxylin or Mallory's simple iron hæmatoxylin.
9. Thorough washing in running water three to six hours.
10. Stain in 1 per cent. Ehrlich's neutral red or Bordeaux red. (This solution must be kept neutral. When it becomes acid it must be neutralized with alkali.) After staining with iron hæmatoxylin the differentiation must be continued until the protoplasm is colorless. The amount of red in the preparation must be controlled under the microscope.

Parasitic bodies in cancer and yeast organisms in the tissue are stained yellowish-red to coppery-red; nuclei, blue-black; connective tissue structures, brilliant red. Plimmer's description of the bodies is as follows:

"The parasites, as they most often occur, are round bodies of very diverse sizes, from 0.004 mm. to 0.04 mm. or even more in diameter. There is a central portion which I shall call here for convenience the nucleus, although there is nothing in this central portion in common with the biological nucleus, which is generally round, but which may be irregular in shape; around the nucleus is a layer of protoplasm, and outside of this is a capsule. This nucleus differs in its microchemical reactions from the nucleus of the cell; it takes, with the Ehrlich-Biondi solution, a copper-red color; with thionin, a dark purple; and with (1) of the double hæmatoxylin stains, described on page 440, it also takes a copper-red color, quite different from the red of either the protoplasm or the fibrous stroma; and with (2) it takes a dark claret color, again darker than that of the protoplasm or stroma. With the Ehrlich-Biondi stain it reacts somewhat like the nucleolus of the cancer-cell nucleus, but the color of the latter is much brighter; moreover, with many other stains—such as aniline-blue or *bleu de Lyon*, anilinegreen—it does not, as does the nucleolus, lose the stain when washed with spirit, but retains it even after immersion. This is to be remembered against the assertion that these bodies are aberrant nuclear structures. In very perfectly fixed specimens a lighter spot can be observed in the nucleus, which is not visible in fresh specimens; but in fresh specimens the nucleus appears to be larger than in fixed ones. It is to be noted that the nucleus is practically refractory to hæmatoxylin used in the strongest manner possible, when this is done by the ordinary methods. This, again, is very important in connection with the statement that the bodies are nuclear structures.

"The layer of protoplasm around the nucleus is generally homogeneous, and stains much less deeply than the nucleus. With alcohol fixation, again, the phenomenon of metachromatism is sometimes seen, the protoplasm staining blue with the Ehrlich-Biondi mixture. When the parasite has attained a certain size a rayed appearance is often seen in the protoplasm, but I have never seen this in the fresh state, and think it may be due to the fixative.

"The capsule is a very well-marked structure, which can be seen well both in the fresh as well as in fixed specimens. That this capsule is a part of the parasite can be demonstrated by the fact that it can sometimes be seen folded over on itself, and sometimes from fixation it shrinks away from the protoplasm of the cell, and also the parasite can sometimes be seen free in an alveolus with its capsule around it. It stains more definitely than either the nucleus or protoplasm; with the Ehrlich-Biondi mixture it is a brighter red than either of the other structures; with thionin it is darker; with hæmatoxylin, acid fuchsin, and orange it is a clear, bright red, and with hæmatoxylin and Bordeaux red it shows best as a very bright red line.

"This description applies to the forms most commonly met with, but there are some other forms which are met with, especially in cancers of rapid growth; they are, however, rare, so I will merely tabulate them:

"1. The form described above, in which the nucleus has attached to it a small body with similar reactions—*i. e.*, reproduction by budding.

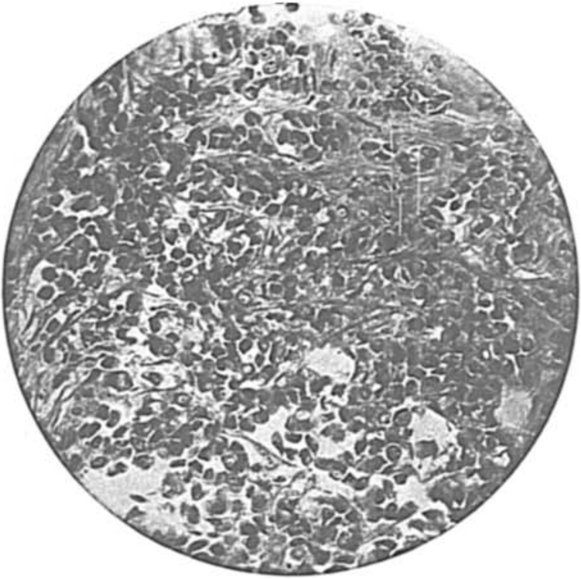
"2. A very small form, consisting of a capsule with a small central dot, staining similarly to the nucleus of the larger forms.

"3. A larger form, consisting of a capsule in which are two large dots in one diameter, and two smaller dots in another diameter at right angles to the first.

"4. A larger form again, consisting of a fine capsule, in which are a number of fine dots, not arranged systematically.

"5. A still larger form, consisting of a well-marked capsule, in which is a central nucleus with generally six smaller dots arranged around it."

Plimmer states that during a period of six years he has examined microscopically 1278 cancers. These did not include any cases of sar-



Section of rapidly growing carcinoma of breast. (Original preparation from Plimmer stained with Plimmer's method.) Nearly every cell contains a Plimmer body (inter-cellular protozoa). (Low Power.)

coma. In 1130 of these cases he found parasitic bodies which come under the heading of those just described by him. His list included cancer of the breast, skin, including tongue and penis, uterus and vagina, stomach and intestines, liver, pancreas, lung, bladder, and glands. The parasites were not found in all portions of the cancer, but were usually present at the growing edge, and in the degenerated portions of the growths they were absent. They appear only in the bodies of active cells, and not in those which show retrograde or degenerative changes. They may be found free lying between the cells and having the same reactions as those which are included within the cell protoplasm. They may also be found in leucocytes. They did not appear in anything like equal numbers in all cases. Generally speaking, in most cancers they were comparatively few in number, but in some very rapidly growing cancers they could be found in enormous numbers, as in the case which he illustrates. He states that in his entire list he has only nine in which there were a large number of parasites; but in these cases there was scarcely a cell which did not contain one or more or even as many as sixty parasites. He states that he has likewise examined a large number of other pathological conditions with a view of determining whether similar bodies could be found. These structures included gummata, histoid tumors of all kinds, tubercular growths, glanders, actinomycotic growths and tissues which had been irritated, as well as normal tissues, and concludes by stating: "I have never in man, in any instance, seen any intracellular or extracellular bodies which were like the parasitic bodies described above, or which had their reactions."

The sixth portion of Plimmer's article is devoted to a description of attempts to cultivate the parasitic inclusions which he describes, and states that in the last case which he has investigated, and which contains a large number of parasitic bodies, he was able to isolate the organism which, in certain animals, was capable of causing death by the production of tumors in various parts. The primary growth was a carcinoma of the breast taken from a woman, aged thirty-five years. It had a history of only two months' duration, and was growing rapidly at the time of operation. Prof. Plimmer has been kind enough to present me a section from this case, a photograph of which is shown on Plate VIII. In cultivating the organism he employed a medium, the basis of which was an infusion of cancer prepared according to the formula employed for ordinary bouillon, and to this was added, after careful neutralization, 2 per cent. of glucose and 1 per cent. of tartaric acid. Into this were introduced small pieces of the growth, cut with all possible precaution against contamination, and the inoculated tubes were placed under anaërobic conditions. After three to five days in three of the flasks inoculated he found a culture of an organism which undoubtedly belongs in the yeast group. We are again indebted to Prof. Plimmer for a culture of this organism. When grown upon ordinary culture media it is that of an ordinary yeast (see Plate VII., Fig. 1). Its histological characteristics are shown in Plate VII., Fig. 3.

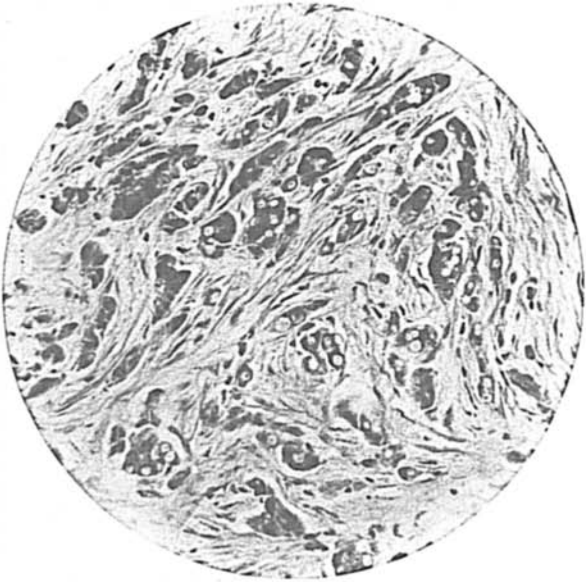
I wish to thank Prof. Plimmer for the courtesy which he has constantly shown our institution, both by communicating to us the result of his more recent work and by supplying us with sections and cultures.

Beside Plimmer, several observers claim to have been successful in the cultivation of yeast organisms from cancer. Kahane obtained a culture of blastomycetes from a cancer of the uterus; Mafucci and Sirleo have obtained cultures from malignant tumors, but their results were mostly negative; Corselli and Frisco report a case of sarcoma of the mesenteric glands, from which they isolated a blastomyces. They obtained the organism during the life of the patient, and after death from the fluid in the abdominal and thoracic cavities. This is apparently a case of true yeast infection. Curtis has reported a case of myxomatous tumor in man, from which he obtained a culture of a blastomyces, which after inoculation produced a similar tumor in a rabbit. Sawtschenko, who published an article in the *Bibliotheca Medica*, 1895, on "Sporozoa in Tumors," states that on a further consideration of his specimens he is now inclined to believe that they are altered yeast organisms. Anna Steckson, in an article on "The Blastomyces of Curtis and its Relation to the Etiology of Tumors," Stockholm, 1900, states that she has cultivated blastomycetes from five cases of carcinoma. The organisms grew on all the ordinary forms of culture media.

In reviewing Plimmer's work the following points present themselves:

1. Can the bodies differentiated by Plimmer's methods and found by him in a large number of cancers be demonstrated in all cases of carcinoma?
2. Is their morphology constant and do they present characteristics by which they can be invariably recognized?
3. Can they be explained as changes in the protoplasm due to degeneration or other causes?
4. Are there morphological and biological grounds for believing that they are altered blastomycetes?
5. Do they bear any relation to the parasites described by other authors—*i. e.*, Russell's bodies and the various cell inclusions interpreted as protozoa?

To determine whether Plimmer's bodies are constant in all malignant tumors a systematic investigation was undertaken immediately after the publication of Plimmer's article. The writer obtained from Prof.



Section of rapidly growing carcinoma of breast. (Case 113, Buffalo.) The magnification is the same as Plate VIII. Nearly every cell contains a parasite. Many cells contain more than one.

Plimmer sections from the case he had published, in which the organisms were stained, which were used for comparison, and a detailed account of his staining method was obtained in person. It will be noted in referring to Prof. Plimmer's article that he failed to mention in the description of his method for the demonstration of the bodies the peroxide treatment of the sections which is step six of the method. This is an extremely important point, and may have led to negative results with those who have adhered strictly to the method as published in the *Practitioner.* We do not know how Plimmer came to overlook this step in his publication, or how important he regarded it, but in our experience it seems to be distinctly fundamental, as the tissues do not take either the iron hæmatoxylin or the red satisfactorily when it is omitted.

During the past two years a large number of tumors, both malignant and non-malignant, as well as tissues not related to tumors, have been hardened and stained according to Plimmer's methods, and a large number of sections from each case have been carefully scrutinized. *The results of our observations completely substantiate Plimmer's claim that these bodies are present in all carcinomata.*

In tabulating the results we have used the terms "present" and "absent," and under "present" have classified the number of bodies encountered as "few" and "occasional." Under "few" is meant only one or two organisms to a section, or less than one organism to a section. "Occasional" means eight or ten or more to a section. The results of our investigation in this direction are shown by the accompanying table:

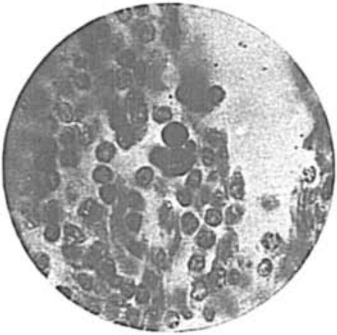
Typical Plimmer's Bodies, Internuclear Protozoan Forms, and Russell's Bodies.

	Present.		
	Few.	Occasional.	Absent.
Soft carcinoma of breast, primary, skin unbroken	8	5	...
Adenocarcinoma of breast, primary, skin unbroken	1	...	...
Soft carcinoma of breast, with metastases in axillary lymph nodes	2	1	...
Recurrent soft carcinoma of breast	...	2	...
Ulcerating scirrhous carcinoma of breast	1	...	...
Large fungating carcinoma of breast	...	1	...
Rapidly growing primary soft carcinoma of breast contained Plimmer's bodies in great number; nearly every cell contained one or more well-defined bodies except in the central degenerated portions of the tumor. See Plate IX.	...	1	...
Scirrhous carcinoma of the breast, material from which was not fresh	...	...	1

	Present.		
	Few.	Occasional.	Absent.
Carcinoma of stomach	...	1	...
“ “ “ metastases in liver	1	...	...
“ “ “ “ in mesenteric lymph nodes	...	1	...
“ “ pylorus	1	...	...
“ “ “ metastases in mesenteric and omental lymph nodes	...	1	...
Adenocarcinoma of rectum with metastases	...	1	...
“ “ of colon	1	...	...
“ “ of appendix, with mucoid involvement of peritoneum; in undegenerated portions of tumor	1	...	...
Mucoid carcinoma of cæcum, involving peritoneum, in undegenerated portions of tumor	1	...	...
Adenocarcinoma of cæcum	1	...	...
Carcinoma of gall-bladder	2	...	...
Secondary carcinoma of liver, ox, only organ examined	1	...	...
Carcinoma of omentum	1	...	...
Adenocarcinoma of uterus	1	...	...
Solid soft carcinoma of uterus	...	1	...
Adenocarcinoma of ovary	2	1	...
“ “ “ protozoan forms in large numbers. (See Plates XI. and XII.)	1	...	...
Case of general carcinosis involving liver, lung, heart, and kidneys, a very acute case	1	...	...
Primary adenocarcinoma of kidney, pig	1	...	.
Hypernephroma from man	2	...	...

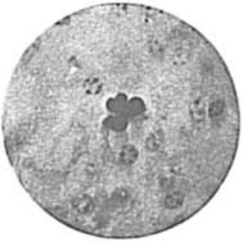
RUSSELL'S BODIES, MODIFIED PLIMMER'S BODIES, PROTOZOAN FORMS.

	Present.		
	Few.	Occasional.	Absent.
Squamous epithelioma of skin	...	1	...
“ “ “ cheek	...	3	...
“ “ “ lip	...	3	...
“ “ “ tongue	...	2	.
“ “ “ penis	...	1	...
“ “ “ cervix	...	2	...
“ “ “ vagina	...	1	...
Squamous epithelioma of orbit, cow; a few atypical bodies giving the reaction of Plimmer bodies, but much larger. (See Plate XIII., Fig. 1)	1	..	...
Multiple sarcoma of skin	1	...	...
Melanosarcoma of skin	3	...	...
Large spindle-celled sarcoma	1	...	...
Spindle-celled sarcoma, metastases in myocardium, horse	1	...	...
Sarcoma of kidney, rooster	1	...	...
Recurrent sarcoma of thigh	1	...	...
Sarcoma of breast involving thoracic wall, dog	1	...	...
Angiosarcoma of brain	1	...	...



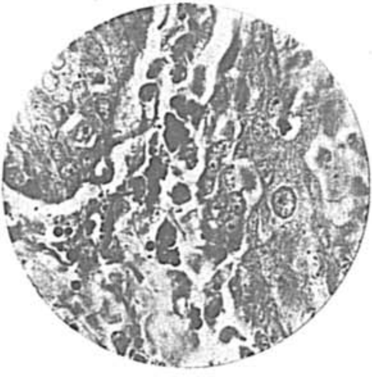
Russell's bodies from carcinomatous lymph node.
(Oil immersion.)
(Plimmer's method.)

1.



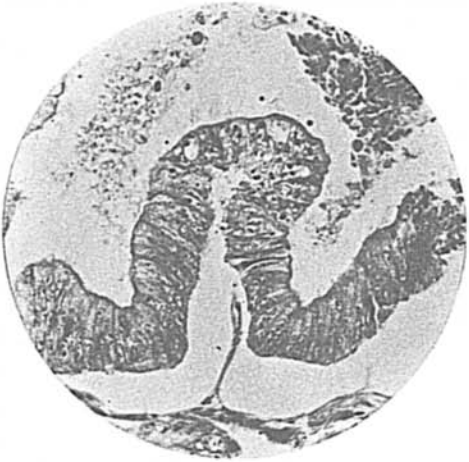
Russell's bodies from enlarged regional lymph node. (Carcinoma of breast.) (This node was used to inoculate Dog 18.)
(Oil immersion.)
(Plimmer's method.)

2.



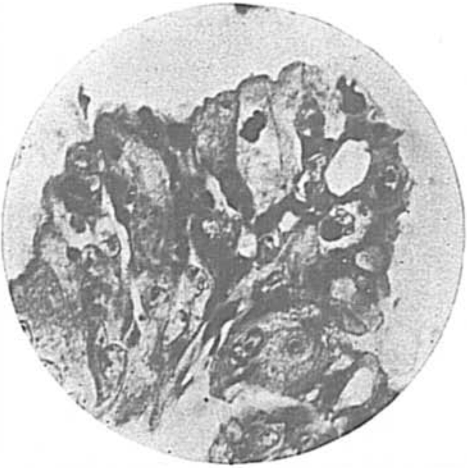
Groups of Russell's bodies from margin of an epithelioma of skin.
(Oil immersion.)
(Methylene blue.)

3.



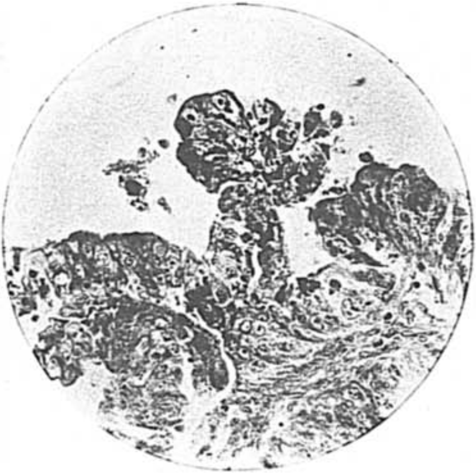
1.

Adeno carcinoma of ovary. Localization of young parasites (Russell's bodies) in the apices of the papillæ. (Plimmer's method.) (M. P.)



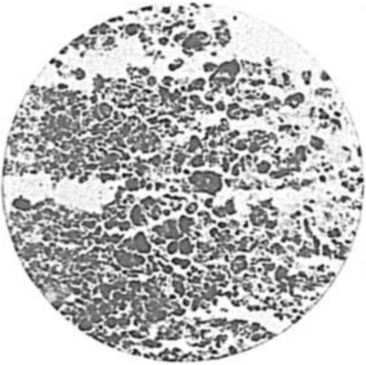
2.

Parasites with pseudopods extended. Same tumor. (Oil immersion.)



1.

Adenocarcinoma of ovary with inter-cellular parasites. (M. P.) (Plimmer's method.)



2.

Free parasites in cystic cavity of tumor. (M. P.) (Plimmer's method.)

	Present		
	Few.	Occasional.	Absent.
Round-celled sarcoma of ilium	1	...	...
Malignant lymphoma	1	...	...
Slowly developing lymphoma	1	...	...
Myxoma of breast	1	...	...
" from pylorus of tiger shark (Galeocerdo tigrinus)	1	...	...
Osteosarcoma	1	...	...
" " of jaw, horse	1	...	...
Adenofibroma of breast, rapidly growing, both breasts involved (see text)	1	...	...
Lipoma of abdominal wall	...	...	1
Fibroma from side of sucker (Chasmistes)	...	...	1
" of musculospiral nerve	...	...	1
Colloid struma	...	2	...
Tuberculosis of breast	...	...	1
Chronic cystic mastitis	...	...	1
Syphilitic lymph nodes[1]	...	1	...

In examining the above table it will be seen that in all cases of carcinoma investigated by this method, with the exception of one which was not fresh, Plimmer's bodies were uniformly present. Likewise in all of these tumors Russell's bodies were numerous, especially about the periphery of the tumors and *in the adjacent lymph nodes, even when these contained no epithelial deposits* Such groups of Russell's bodies are shown on Plate X. One tumor, an adenocarcinoma of the ovary, beside containing typical Plimmer's bodies, contains a large number of cellular inclusions resembling Russell's bodies, and many larger forms which show evidence of previous amœboid movement. These are the so-called protozoan forms of the earlier writers. It will be noted on Plate XI., Figs. 1 and 2, and Plate XII., Fig. 1, that these organisms have infected the epithelium at various points, and as a result of the infection a proliferation has occurred which has thrown the epithelium into folds giving the characteristic papillary appearance of such tumors. As a result it is to be noted that the parasites are present in greatest number at the apices of the papillary projections. The fluid in the cavity of this tumor appears to be nearly a pure culture of organisms,(Plate XII., Fig. 2). This tumor was hardened immediately after the operation in sublimate, and was brought by the writer from Dresden, where it was obtained over three years ago. One of the cases in which Plimmer's bodies were found was a rapidly growing adenofibroma of the breast. *This case presented no histological evidence of malignancy, but occasional Plimmer's bodies were found lying in the stroma and between the cells of the acini, as well as a few in cells. The patient developed a similar growth in the remaining breast,*

---

[1] The organisms found in syphilis closely resemble those of sarcoma. This phase of the question will be reported upon later.

which was likewise operated upon after it had reached a large size, and it likewise contained a few Plimmer's bodies.

Beside these cases of carcinoma fourteen cases of squamous epithelioma were examined, and all of these contained *atypical bodies which gave the staining reactions of Plimmer's bodies, but were much larger and did not contain the characteristic central bodies.* The appearance of the cell inclusions from one of these cases is shown in Plate XIII., Fig. 1. The remainder of the list contains fifteen cases of sarcoma—eleven from man and four from animals—*all of which contained the small forms of the organism within the nuclei. Plimmer's bodies are rare, and not so well defined as in carcinoma.*

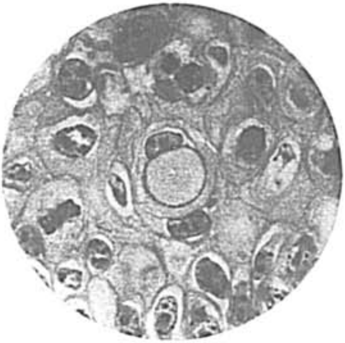
In comparing our findings with those of Plimmer, it will be seen that the results obtained by us completely substantiate his claim that typical bodies of the nature which he has described are of practically constant occurrence in carcinoma and sarcoma. Our list is not nearly so large as Plimmer's, as we have devoted our principal efforts to the experimental side of the problem, and simply wished to confirm or disprove Plimmer's results. We have not considered it necessary to examine a greater number of tumors than those employed for experimental purposes. Beside the tumors enumerated in this list we have examined a large number that were fresh, and have been able invariably to demonstrate the parasites in the fresh condition.

The recognition of the nature of these bodies and the demonstration of their presence in 88 per cent. of 1278 cases is a feat so colossal that it places beyond any question of doubt the significance of these bodies in the cancerous process. Not only should the credit of this performance be freely accorded to Plimmer, but we must also state that the acquisition of his staining method has thrown an entirely new light upon the animal experiments which we shall describe later, and has been one of the most important factors in elucidating the entire problem.

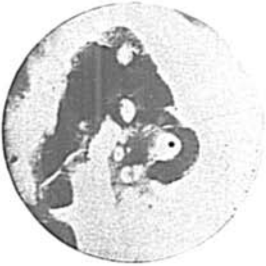
As to whether the morphology of these structures is sufficiently constant to enable their ready recognition the following can be stated:

The bodies may be demonstrated in the fresh state, in which case it is a matter of considerable difficulty to distinguish them from fat droplets, which they somewhat resemble, but which have a higher refractive index and do not contain a central structure. The most minute form of the organism might be confused with cocci. In the fresh state the quarter-grown and half-grown forms appear either as spherical bodies with very delicate outlines and containing one or two colorless granules near the centre, or as similar structures of oval form. They may be either intracellular or extracellular. Occasional forms will be met in which the pale structure of the body is projected in the form of a pseudopod; in which case the granules may remain in the larger portion of the structure or one or two may be found in the projection.

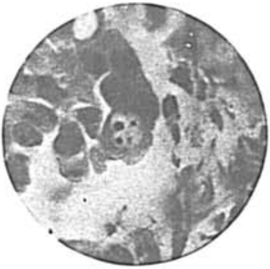
PLATE XIII.



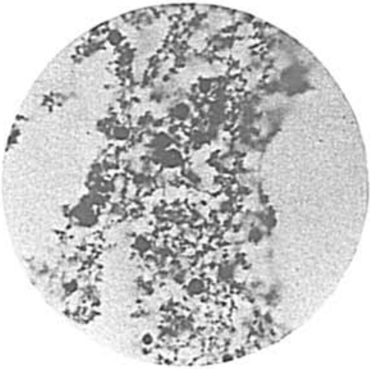
1.



2.

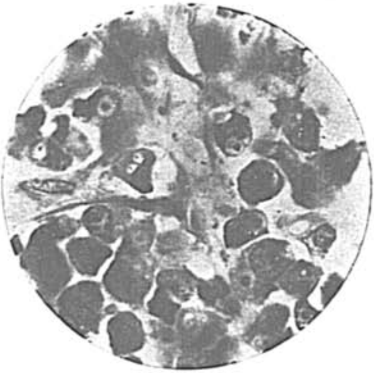


3.

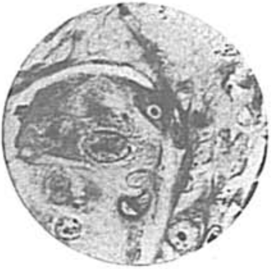


4.

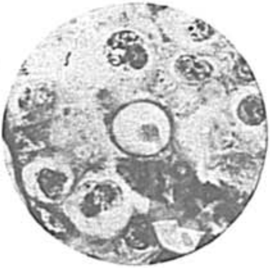
PLATE XIV.



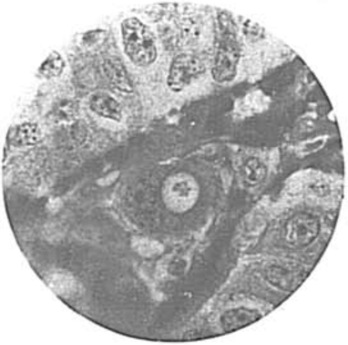
1.



2.



3.



4.

These forms closely resemble the bodies shown in Fig. 2 of the article by Sjöbring in the *Centralblatt für Bacteriologie*, vol. xxvii.

We have never been able to detect any change of form in the intracellular bodies, although scrapings from carcinoma in which they were found have been exposed to various procedures. The extracellular bodies may be occasionally induced to change their form by placing the preparation in a thermostat. In this case we have repeatedly observed that bodies which are spherical changed on the warm stage after a period of some hours, and projected longer and shorter processes. Attempts to stain them in the fresh state have met with varying results. They apparently take on Sudan III., but Dr. Clowes, the chemist of the laboratory, states that this is not a genuine staining reaction.

We find that the organism of vaccine may be stained in the same manner, and we are informed that others have employed Sudan III. as a stain for amœbæ.

Attempts to fix the organisms by heat gave unsatisfactory results, owing to the distortion which they undergo. If cover-slips be made and dried in the air the small, spherical bodies will stain with any of the aniline dyes. The larger forms, however, remain unstained, and appear as spherical or oval clear spaces in the stained material of the cover-slip. The best means to fix them is to make rapid smears after the manner employed in making blood-slips and drop the wet cover-glasses into warm Hermann's fluid or sublimate. They may then be stained with Plimmer's method. We have likewise found that pouring peritoneal fluid which contained them into warm Hermann's fluid or sublimate and treating the coagulum after the manner in which tissue is manipulated gives excellent results, the organisms retaining their form and characteristic appearance. (Plate XIII., Fig. 4.)

The best method for preserving the extracellular forms is by hardening masses of tissue which contain them. An excellent method for hardening the organisms with the pseudopodia projected has recently been described by Eisen in his very excellent article in the *Medical Record* of July 7th of this year. Dr. Eisen has been kind enough to forward us some of his sections, and we can heartily indorse what he has described in epithelioma.

In sections hardened in Hermann's fluid and stained with Plimmer's method the bodies correspond very accurately to the description given by Plimmer. Some of the different types which we have observed are shown on Plate XIII., Figs. 1, 2, and 3, and Plate XIV., Figs. 1, 2, 3, and 4. As to the staining qualities of the organisms, we find that they closely conform to those given by Plimmer—that is, that the central body and capsule of the structure take on a coppery-red or pale rose color. In the case shown on Plate XIII., Figs. 2 and 3, we have succeeded in staining the central bodies with the nuclear stain, and in some of the cell inclusions only the central portion of the central body takes the nuclear stain, and is then surrounded by a layer of protoplasm, which takes the rose or pink color.

The organisms may appear singly in the cells, or even in large numbers. (Plate XIII., Fig. 3.) We have detected as many as fourteen in one cell. There is no difficulty in detecting the structures in properly hardened and stained material. They can be readily distinguished from vacuoles in the protoplasm by the well-defined capsule and central bodies, their relative uniformity in size, and occasional presence between the cells.

Summing up this phase of the question, it can be unhesitatingly stated that Plimmer's bodies present a characteristic appearance and can be readily differentiated from cell degenerations of the usual type and other structures which they might resemble.

To determine whether or not Plimmer's bodies could be due to cell degenerations, we have carefully examined a large number of sections of well known pathological conditions of known cause, such as tuberculosis and certain bacterial infections, and a large number of animals inoculated with various pathogenic yeasts, and we can state that we have never detected any changes in the epithelial or other cells in this class of diseases which could be confused with Plimmer's bodies.

In attempting to determine the nature of Plimmer's bodies, the fact becomes immediately patent, in reviewing the literature, that many observers have depicted these structures. Scarcely an article has appeared in which the observer has not, more or less accurately, illustrated cell inclusions which are no doubt the characteristic bodies of Plimmer. The slight variations which are encountered in these illustrations are very likely due to the different methods of hardening and staining employed by the different investigators, and the usual subjective equation incident to all illustration by drawings. Figures which are unquestionably intended to illustrate bodies of this nature may be found in a publication of Sawtschenko, *Bibliotheca Medica*, 1895, Abtheilung, D. ii., Heft 4. Those which most characteristically represent Plimmer's bodies are Figs. 18, 19, 30, 51, 57, and 60. In an article published by Jackson Clark, *Centralblatt für Bacteriologie*, vol. xvi., Fig. 6 of Plate III. is a most typical representation of these bodies, and, in like manner, an investigation will reveal more or less characteristic illustrations in the publications of the majority of investigators.

Of especial interest in this connection are the illustrations of Sjöbring accompanying his most recent publication in the *Centralblatt für Bacteriologie*, vol. xxvii. Fig. 1 of this article represents a section through the wall of a *vasa efferentia* in the epididymis. In the protoplasm of the epithelium are shown three typical Plimmer's bodies. This illustration is especially interesting, as the author states it is taken from the epididymis of an animal from the neighborhood of a fragment of sterile carcinoma which had been implanted in the testicle.

It will, therefore, be seen that many of the observers who have described cell inclusions, in the belief that they were protozoa, have unquestionably seen typical Plimmer's bodies, and it would appear as if Sjöbring, in his most recent publication, was dealing with bodies of at least a very similar appearance.

In all the carcinomata which we have investigated we have found that Russell's bodies could be detected, especially about the periphery of the tumor and in the enlarged regional lymph nodes, even when these were not carcinomatous. In sections stained with Plimmer's method they take on a dense blue-black, but by modifying the stain they can be made a brilliant red, which gives them much the same appearance as they possess when stained with fuchsin. In one case we have succeeded in staining the central bodies of the Plimmer inclusions, and are able to trace a direct morphological relation between Russell's bodies and Plimmer's bodies. In one case of adenocarcinoma of the ovary, which contained a large number of Russell's bodies and protozoan forms, the bodies which correspond to Plimmer's bodies were more or less atypical, probably owing to the hardening agent (sublimate).

From the above it will be seen that Plimmer's bodies are not new structures, but that his hardening and staining methods give them a more characteristic appearance and probably differentiates them in a large number of cases in which, when treated by the ordinary staining methods, they would be invisible.

In form the bodies are almost always round or oval. Occasionally they occur in pairs, in which case they are usually smaller than the single bodies, and present an appearance which is highly suggestive of some form of division. Plimmer describes a budding form of the organism, which, in our experience, is very rare. We have found only one or two bodies which conform to this description.

The fact that certain observers have succeeded in obtaining pure cultures of blastomyces from carcinoma, and the significant experiments of Sanfelice, naturally raise the question as to whether these organisms can be detected in carcinomata. In the examination of our cases we have thus far failed to find any structures which we were willing to consider blastomycetes. During the last two years we have carried out an elaborate investigation of pathogenic yeasts, including Plimmer's organism, Sanfelice's neoformans and lithogenis, the pathogenic blastomyces isolated from a skin lesion by Hektoen, and a similar organism isolated by Gilchrist and Stokes.

A summary of the results of a long series of comparative inoculations with these organisms, as well as a careful morphological comparison, will form the subject of a future publication from this laboratory. The details of the results will not be entered into here. Suffice it to say that we have never detected a sufficient transformation in the organisms injected to give them an appearance identical with the Plimmer bodies of carcinoma. The nearest approach to such a result is shown in Plate XV., Figs. 1 and 2, which represents yeast cells (Plimmer's) in the lung of a guinea-pig, hardened and stained by his method.

Thus far the distinguishing feature which we have always observed has been the double contoured capsule of the yeast organism, which Plimmer's bodies do not possess. At the same time we have occasionally noted yeast organisms with a single contoured capsule; but in these cases they never presented an appearance which could be confused with a typical Plimmer body. Plate XV., Fig. 1, shows a yeast organism with a single contoured capsule, which, it will be noted, contains no central body. *From a morphological stand-point we have been unable to demonstrate the identity between Plimmer's bodies and transformed yeast organisms.*

The culture experiments in this laboratory have been of a uniformly negative nature. An elaborate bacteriological investigation was carried out by Dr. Pease, former bacteriologist to the laboratory, at the time the tumors were being investigated for the presence of Plimmer's bodies, and in only one case did he succeed in obtaining a culture of a blastomyces. In order, if possible, to find a suitable medium for the blastomyces, in case the negative results were due to unsuitable substrata, we prepared and employed sixty-four different varieties, which it may be of interest to enumerate. In this list the term + is used to indicate acidity, and — alkalinity. The figures indicate the units of acidity and alkalinity recommended by the bacteriological committee.

Bouillon neutral.
" + 15.
" + 5.
" — 15.
" sugar (free).
" glucose.
" lactose.
" saccharine.
" glucose—tartaric acid.
" pig liver.
" " liver—cancer.
" " kidney.
" glucose—cancer.
" " —tartaric acid—cancer.
" dog—cancer.
" asparagin.
" potash soap.
" human glucose—neutral.
" tartaric acid—glucose (human).
" fucus crispus.

Milk.

Serum bovine.
" human.
" dog.
" ascitic.
" hydrocele.
" abdominal fluid.

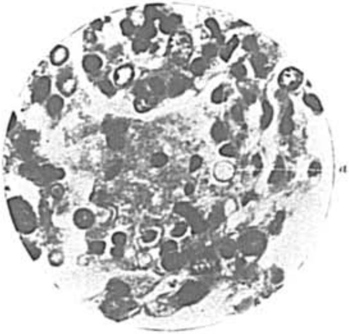
Urine.

String-beans in aqua dest.
" " " Niagara water.
Bean stems—glucose—tartaric acid.

Beerwort.

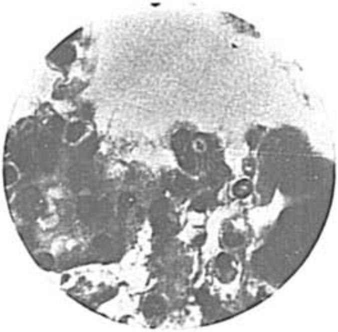
Potato infusion.
Potato.

PLATE XV.



1.

Plimmer's yeast in lung of guinea pig. Hardened and stained according to Plimmer's method. At *a*, an organism with single contour. (Oil immersion.)



2.

Field from same section as Fig. 1. Yeast with thick capsule in act of budding. Central bodies. (Oil immersion.)

Agar neutral.
" + 15.
" — 15.
" potato.
" glucose.
" glucose—tartaric acid—cancer.
" beerwort.
" serum.
" hay.
" glucose—hay.
" glycerin.
" fucus crispus.
" cabbage water.

Gelatin neutral.
" 15.
" potash soap.
" beerwort.

Hay infusion.
" glucose water.

Sugar water.
Glucose water.

Fucus crispus water.
" " hay infusion.

Cabbage water.

Lettuce water.

Bread.

Human fat.

Casagrandi.

Nahrstoff (Hayden).
" " soda.

In our experimental inoculations of animals we have usually been able to obtain the blastomyces by cultivation after the inoculation. The majority of lesions which we have produced have been those of typical blastomycetic mycosis with abscess formation, or characteristic granulomata containing the organism.

Having thus determined to our satisfaction that the yeast organism was not the essential cause of carcinoma and sarcoma and could not be confused with the organism we had already observed in fresh scrapings of carcinoma and in our experimental animals, and having already convinced ourselves of the identity of the organisms found in the fresh state and the forms known as Russell's bodies, Plimmer's bodies, and protozoan forms in the tissues, our attention was called to the possible relation between these inclusions in cancer and the vaccine bodies observed after inoculation of the cornea with vaccine virus, by an article by Dr. C. Gorini, in the *Centralblatt für Bacteriologie*, September, 1900, vol. xxviii., No. 8/9, entitled "Ueber die bei der mit Vaccine ausgeführten Hornhautimpfung vorkommenden Zelleinschlüsse und über deren Beziehungen zu Zellinklusionen der bösartigen Geschwülste." We had already noted the similarity of Russell's bodies to the illustrations of inoculated corneas, and it remained for us to harden and stain the cornea of rabbits which had been inoculated with vaccine virus after Plimmer's method. The result of our comparative test in this case shows not only that the half-grown form of the vaccine organism is very closely related in appearance to the bodies of Plimmer, but that the bodies of Russell in carcinoma and the protozoan forms in carcinoma, all have their prototypes in the various stages of development of the vaccine organism. (See Plate XVI., Figs. 1 and 2.)

This striking confirmation of Gorini's observation was more than doubly confirmed when, after the recent announcement of Dr. M. Funk, in the *British Medical Journal* of February 23d of this year, on the cultivation of the vaccine organism, we repeated his experiment and found that the organism of vaccinia, while undergoing development, shows essentially the same phases we had already noted in the organisms observed in fresh scrapings of cancer and in the peritoneal fluid and blood of cancer cases.

Having thus convinced ourselves that the organism with which we were dealing was a protozoon belonging in the same group with the vaccine organism, it remains to be seen what evidence can be produced to show that these protozoa, which are a constant occurrence both in the fresh material of cancer and which we had cultivated and identified in the tissue under the form of Russell's bodies, Plimmer's bodies, etc., are the cause of cancer.

We have found it necessary to carefully restain the tissues of all our earlier animals with Plimmer's method, and, as this work is not yet complete, we deem it advisable to withhold the complete series until we are in a position to consider certain phases of the question which will require an investigation of tissues from other diseases, which are possibly protozoan infections.

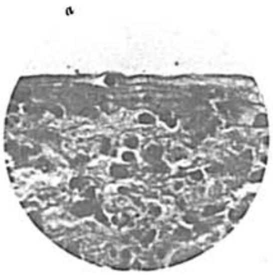
That the protozoon of cancer is capable of producing even in man lesions of a very different nature from infection of the epithelium, seems to be strongly indicated by the following observation:

Case 108. Mrs. E., aged forty-five years. Well-developed cancer of right breast, with axillary lymph nodes, operated upon February 5, 1901, at Buffalo General Hospital by Dr. Park. The case in question attracted our attention because of a pigmented pustule beneath the skin of the right axilla. The clinical notes in brief are as follows:

Three years ago observed a retraction of the nipple. Six weeks ago noted a well-defined tumor in the right breast. At the same time a small nodule was felt beneath the surface of the skin of the right axilla. This developed in a region which was subject to irritation by the edge of the patient's corset.

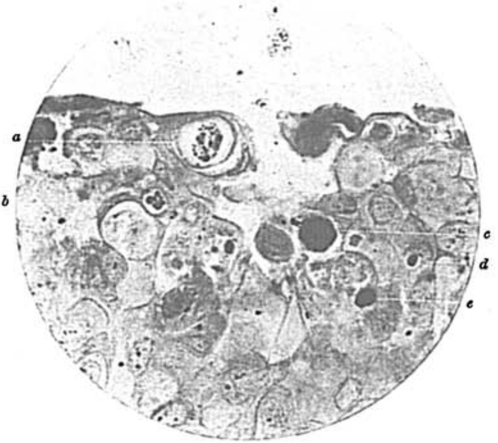
In removing the tumor Dr. Park carried a flap up into the axilla, removing the axillary lymph nodes and including the pustule above mentioned. The tumor reached the laboratory one hour and a half after the operation. An inspection of the tissues removed shows them to consist of an enlarged right breast with a thickened and retracted nipple. The flap of skin removed is included between two curved incisions. At a distance of about five inches from the nipple there is an elevation of the skin somewhat larger than a pea. The skin cover-

PLATE XVI.



1.

Recent vaccination of cornea (third day). At *a*, young vaccine organism (analogous to Russell body) entering epithelial cell of cornea. Oil immersion,) (Plimmer's method.)



2.

Section of vaccinated cornea (seventh day). (Plimmer's method.) At *a* and *b*, bodies analogous to Plimmer's bodies; *c* and *e*, younger forms of parasite. At *d*, a small organism within the nucleus of an epithelial cell.

ing it is glazed, and on palpation it is found to contain fluid. On opening this pustule a small amount of dirty red fluid escapes. Examined in the fresh state with the microscope this material is found to consist of leucocytes, red blood-corpuscles, and a large number of cells, from five to ten times the diameter of a red globule, possessing coarse and fine granulation. Some of them contain highly refractive bodies somewhat finer than ordinary cocci, but the greater number have embedded in their protoplasm poorly defined, hyaline, pale-green, spherical bodies, somewhat smaller than red blood-corpuscles. When treated with acetic acid these hyaline bodies and the granules of the cells become more distinct, and nuclei may be made out. Beside these cells are a large number of ordinary pus cells, which form the principal constituent of the fluid.

On cutting into the breast it is found to consist for the most part of fat, through which runs sharply-defined bands of carcinomatous tissue. At no point in the structure is a well-defined tumor mass. From the most vascular portion of the carcinoma fresh scrapings were made. Under the microscope these scrapings are found to consist of closely packed epithelial cells with vesicular nuclei. A large amount of free fat is found in the preparation, but the cells show very slight evidence of fatty degeneration. Between the fat droplets are a considerable number of small, greenish hyaline bodies, which, in our experience, can be recognized from fat by the slight difference in refractive index and color.

Portions of the tumor were hardened in Hermann's fluid. On examining the axillary fat a group of five or six enlarged lymph nodes may be detected. On dissecting these out and incising them they are found to contain more or less extensive deposits of cancer. Sections of these were hardened in sublimate and Hermann's fluid.

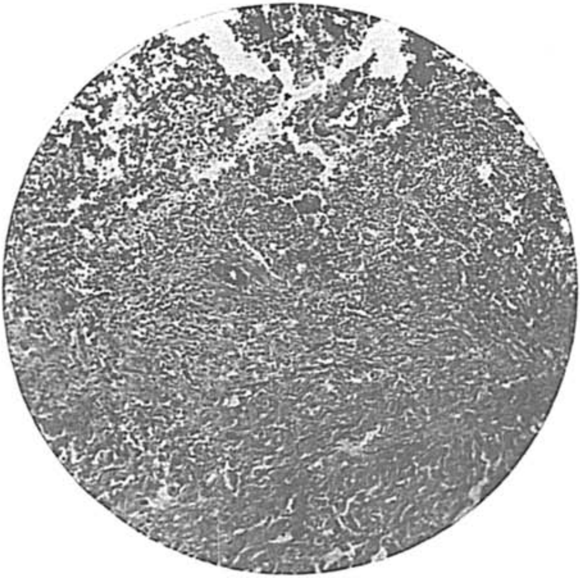
Fresh examination of scrapings from these lymph nodes reveals the presence of bodies which correspond to what we have recognized as the fresh Plimmer body. They are composed of pale fluid protoplasm, and contain a group of fine granules. Their outlines are well defined, and in many cases they appear to be sending out projections. In some of these bodies the protoplasmatic granules show active Brownian movement. An examination of the sections from the tumor and lymph nodes shows the case to be one of soft carcinoma of the breast with metastases in the axillary lymph nodes. Stained with Plimmer's method a considerable number of the various forms already mentioned can be found—Russell's bodies, Plimmer's bodies, and protozoan forms. A section through the pustule hardened and stained with Plimmer's method reveals a most interesting condition. It consists of an abscess in the subcutis. Upon the surface the papillæ of the cutis are normal, but directly above the abscess three or four of the papillæ are seen to be hypertrophied and extend into the subcutaneous tissue. The abscess appears to be made up for the greater part of masses of spherical bodies somewhat smaller than leucocytes. There is no apparent inflammatory reaction about the periphery. The bloodvessels show no engorgement. (See Plate XVII.) Under high dry power the abscess is found to be composed of leucocytes with horseshoe nuclei and a large number of spherical and oval, deeply-stained bodies, which closely resemble large Russell's bodies. (See Plate XVIII.) Beside the leucocytes and these small spherical bodies are a large number of large, oval, and spherical cells with one or two nuclei. Many of these contain deeply-stained spherical bodies, somewhat smaller than the free spherical bodies in the tissue. Others of nearly the same size contain a large number of small hyaline bodies which do not take the stain. These, we are inclined to believe, are the sacs of the parasite. (See Plate XVIII.)

Beside the small forms and large sacs we have found a number of spherical bodies about four times the diameter of the leucocyte, which stain brilliantly with the Bordeaux red of the preparation. In a number of these bodies we have found evidences which are strongly suggestive of segmentation, after the manner recognized in malarial parasites. The bloodvessels within the focus contain, beside red blood-cells, large numbers of the deeply stained oval and spherical Russell's bodies. In some cases the endothelium of the capillaries is greatly swollen and contains one or more of these deeply stained bodies.

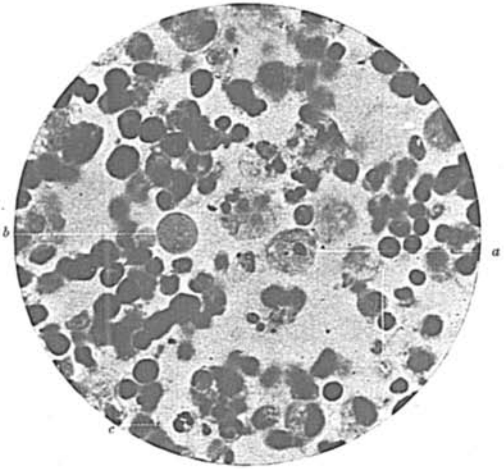
We are inclined to believe from this observation that this pustule is the result of embolic deposit of the parasites from the cancer of the breast, and we would interpret it, pending further observation, especially in the light of our animal experiments, as indicating that the organism of cancer is capable of producing other lesions than that of epithelial infection.

The material with which our animals were inoculated consisted of peritoneal fluid from cases of abdominal carcinosis, fluid from the interior of malignant ovarian cysts, sterile cancer, and dried sterile cancer and lymph nodes rubbed up with salt solution. Some of these latter contained metastatic deposits of cancer, but a number were simply the enlarged lymph nodes, in which we had already detected Russell's bodies. In each case the fresh material was carefully examined before and the presence of parasites was determined. The presence of parasites was likewise verified in hardened sections of the material where this was possible

The following is summarized from the complete list of animals, which will appear in the second part of this communication:



Embolic skin pustule from carcinoma of breast. Deeply stained spherical bodies are protozoa. (Low Power.)



Field from center of skin pustule, shown on Plate XVII. At the center remains of spore cysts. One at *a* contains a single deeply stained body (young parasite). At *b*, a half-grown organism stained red. *c*, a leucocyte. Remainder of field filled with young spherical and oval organisms and polymorph nuclear leucocytes. (Plimmer's method.) (Oil immersion.)

ANIMALS INOCULATED WITH MATERIAL AND CULTURES FROM CARCINOMA AND SARCOMA IN MAN.

2	guinea-pigs	inoculated in the jugular with peritoneal fluid, died in 22 and 19 days respectively.
14	"	inoculated in the peritoneum with peritoneal fluid, died in an average of 58 days.
1	guinea-pig	inoculated in the right eye with peritoneal fluid, died in 10 days.
1	"	inoculated in the jugular with filtered serum, died in 304 days.
1	"	inoculated in the jugular with cancer mush, died in 15 days.
4	guinea-pigs	inoculated in the peritoneum with cancer mush, died in an average of 57 days.
11	"	inoculated in the peritoneum with dried lymph nodes, died in an average of $45^4/_{11}$ days.
7	"	inoculated in the peritoneum with sarcoma, died in an average of $92^2/_7$ days.
2	rabbits	inoculated in the ear vein with peritoneal fluid, died in 93 and 17 days respectively.
1	rabbit	inoculated in the ear vein with filtered serum, died in 164 days.
1	"	inoculated in the peritoneum with cancer mush, died in 292 days.
1	"	inoculated in the peritoneum with sarcoma, died in 278 days.
1	"	inoculated in the mediastinum with sarcoma, died in 41 days.
1	dog	inoculated in the peritoneum with peritoneal fluid, died in 64 days.
1	"	inoculated in the peritoneum with sarcoma, died in 160 days.

ANIMALS INOCULATED FROM ANIMALS INFECTED WITH PARASITES FROM CARCINOMA AND SARCOMA IN MAN.

Pig 23, inoculated in abdominal wall with lymph gland and peritoneal fluid from Pig 16, died in 9 days. Case I., adenocarcinoma of the omentum.

" 55, inoculated in abdominal wall with several nodes from Pig 52, also portion of old original tumor, died in 4 days. Case LVII., squamous epithelioma of skin.

" 72, inoculated in abdominal wall with B-pig 67–a, 2 c.c. node emulsion in water, died in 37 days. Case L., enlarged axillary lymph nodes recurring sarcoma breast.

" 75, inoculated in abdominal wall with $2^1/_2$ syringes B-pig 66–a, nodes powdered in sterile water, died in 59 days. Case L., enlarged axillary lymph nodes recurring sarcoma breast.

" 73, inoculated in abdominal wall with 2 c.c. nodes in water B-pig 64–a, died in 48 days. Case L., enlarged axillary lymph nodes, recurring sarcoma breast.

" 76, inoculated in abdominal wall with A-pig 68–a, nodes powdered in water, died in 17 days. Case LXI., carcinomatous lymph nodes from axilla accompanying well-developed carcinoma of breast.

" 15, inoculated in the abdominal wall with 2 c.c. fluid from peritoneum Pig 13, died in 8 days. Case XXIII., recurrent sarcoma thigh.

The points of interest are that 14 guinea-pigs, inoculated in the peritoneum with peritoneal fluid containing the organism, gave an average length of life of fifty-eight days; 4, inoculated in the peritoneum with cancer mush, gave an average length of life of fifty-seven days; 11, inoculated in the peritoneum with dried cancerous lymph nodes, gave an average length of life of forty-five and four-elevenths days; 6 guinea-pigs, inoculated with peritoneal fluid and lymph nodes from animals which were infected in the above manner, gave an average length of life of twenty-nine days—a little more than half the length of time for the animals inoculated directly from man.

This unquestionably shows the increased virulence of the organisms after passing through one animal. We are continuing these experiments in modified form and shall report upon them later.

The average length of life for rabbits inoculated in various regions with the different forms of material used shows the greater resistance of this animal to infection. In our most recent experiment we have succeeded, by growing the organism in a collodion sac (suggested by Dr. Clowes) in the peritoneal cavity of a rabbit, in so increasing the virulence of the organism that a healthy rabbit inoculated in the ear-vein died of general hæmatogenous infection from the organism after a period of fifteen days. (Rabbit 56 of the list.)

It will be shown from these experiments that animals are readily infected when inoculated with carcinomatous material as well as pure cultures of the organism. The peritoneal fluid used in all of these inoculations was bacteriologically sterile, and consisted essentially of a pure culture of the organism. Two animals—one guinea-pig and one rabbit—which were inoculated with filtered serum from which the organism had been removed, gave a respective length of life of 304 days and 164 days. The organs of these animals were free from parasites.

The macroscopical pathological findings in these cases were generally uniform. All the animals were greatly emaciated and presented, on opening the abdominal cavity, collapsed intestines, reddened peritoneum, enlarged peritoneal lymph nodes, and a moderate amount of clear, straw-colored fluid. The lungs were dark red in color, collapsed; heart contained but small amount of blood; the spleen was enlarged and reddened; the liver in many cases was hyperæmic, and the kidneys were generally injected.

One dog (Dog 18) presented a large lymphoma of the spleen. This case will be considered separately. Dog 8, inoculated from sarcoma, Case XXIII., shows typical metastases of sarcoma in all of the regional lymph nodes.

In almost all cases a fresh examination was made of the peritoneal fluid, the organs, and the blood, and, wherever made, large numbers of the parasites could be readily detected, as already described.

Two guinea-pigs and two rabbits, inoculated in the jugular with peri-

toneal fluid, show macroscopical lesions in the lungs very closely resembling those reported in our Pig 1—that is, minute white dots scattered through the pulmonary structure, usually localized in the neighborhood of the bronchi. Sections from these lungs, stained with hæmatoxylin, show the presence of multiple beginning adenocarcinoma of the bronchi.

These we should interpret as beginning adenocarcinoma of the lung in 4 animals, making, with our Case I., 5 animals in which the injection has been followed by infection of the bronchial epithelium. One guinea-pig presents a condition of the lungs and liver which we wish to interpret as *primary carcinoma of those organs,* and 1 dog presents a lymphoma of the spleen, the size of a large hazel-nut, which we also attribute to the inoculation of the animal with dried lymph nodes from a case of carcinoma. The tumor cells in all of these animals contain the characteristic forms of the parasite.

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1. THORACIC ANEURISM. 2. CARCINOMA OF SUPERIOR MAXILLA. 3. CHOLELITHIASIS AND SUPPURATIVE CHOLECYSTITIS. 4. INGUINAL HERNIA OF (*a*) BLADDER; (*b*) CÆCUM.[1]

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1. *Thoracic Aneurism.* This case was an aneurism of the thoracic portion of the left common carotid artery. The patient was a male, aged thirty-nine years, and came under observation in November, 1899. His general condition as regards strength, health, etc., was perfect. Previous history as regards disease of all kinds was absolutely negative.

At the age of twenty-three years, sixteen years before admission to Bellevue Hospital, he had received a pistol-shot wound in the left side of the neck, which healed without complication. Nothing developed until the fall of 1897, when a swelling was noticed under the scar, well down in the neck. From that time the swelling increased in size, slowly but steadily, and with increasing severity of pressure-symptoms, pain, dyspnœa, and coughing, which interfered with sleep and were beginning to undermine the patient's health. Medical treatment had been tried for six months, but without relief.

Examination: On inspection a swelling, about the size of a lady apple, was observed in the left side of the neck close to the sterno-

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[1] Read before the New York Surgical Society, February 27, 1901.